Abstract Book
May 31st- June 3rd, 2023

Wednesday, May 31	Thursday, June 1	Friday, June 2	Saturday, June 3
	9:00-10:30 Translation and therapy I	9:00-10:30 Immunotherapy II	9:00-10:15 Malignant cell biology
	11:00-12:30 Signal transduction	11:00-12:30 Translation and therapy II	10:45-11:45 Molecular mechanisms of disease II
	12:30-14:00 Lunch at Heidemuseum	12:30-14:00 Lunch at Heidemuseum	11:45-12:00 Wilsede Award
14:00-19:00 Shuttles from Handeloh	14:00-15:30 Immunotherapy I	14:00-15:30 X-omics	12:00-13:00 Lunch at Heidemuseum
16:00-17:30 Registration Horse carriages from Undeloh	16:00-18:00 Molecular mechanisms of disease I	16:00-18:00 Biomarkers and diagnostics	13:00 Departure
17:30-18:45 Welcome & Appetizers	18:30-19:00 Hartmut Kabisch Memorial Lecture; K. Welte	18:30-19:15 Invited Lecture II C. Braun	
18:45-19:30 Invited Lecture I A. Roy			
19:30 Dinner	19:00 Barbecue	19:15 Dinner	

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WEDNESDAY, MAY 31st

17.45 – 18.45 **Appetizers** Chair: Olaf Heidenreich

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

Carla Panisello[1], Bueno C[1], Castilla C[2], Aschero R[3], Carcaboso AM[3], de Álava E[2], Menéndez P[1]

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Ewing Sarcoma (ES) is the second most common bone and soft tissue sarcoma affecting children and young adults. The dismal long-term survival in patients with metastatic/refractory disease is driven by the limited response rates to current multimodal therapies and the lack of actionable targets. This highlights the urgent need for developing new therapeutic approaches. Immunotherapy with chimeric antigen receptor (CAR)T-cells directed against a tumour-associated antigen (TAA) is a promising therapy with a safe and efficient profile in B-cell malignancies. However, the development of efficient and safe CAR T-cells for the treatment of ES is still challenging due to i) the low abundance of specific and safe TAAs, ii) and the presence of an immunosuppressive tumour microenvironment (iTME) that compromises CAR T-cells function. Here, we show preliminary data of specific ES-targets with limited expression in healthy tissues, the optimization of in vitro and in vivo cytotoxicity models which mimic the ES-iTME, and the initial assessment of a CAR T cell construct that turns the iTME to an immunologically hot tumour by triggering endogenous T-cell responses that enable epitope spreading.

Exploring the novel function of mutant NPM1 on chromatin

Hannah J Uckelmann[1], Haarer EL[2], Takeda R[2], Hatton C[2], Chen CW[3], Armstrong SA[2]

[1]Department of Pediatric Hematology and Oncology, Clinic for Pediatrics, University Hospital Frankfurt, Frankfurt am Main, Germany; [2]Pediatric Oncology, Dana-Farber Cancer Institute, Boston, USA; [3] Department of Systems Biology, Beckman Research Institute, City of Hope, USA.

Cytoplasmic NPM1 mutations (NPM1c) in AML were first described almost two decades ago and represent one of the most frequently mutated genes in these leukemias. Much effort has been focused on the cytoplasmic functions of mutant NPM1, however, its mechanistic role in leukemia development remains elusive. Especially, how NPM1c expression in hematopoietic cells leads to its characteristic gene expression pattern including many MLL target genes such as HOXA9 and MEIS1. We have recently shown that NPM1c AMLs are highly sensitive to the disruption of the MLL1 histone methyltransferase complex. Small molecule inhibitors that block the interaction between MLL1 and its adaptor protein Menin have been shown to impair binding of MLL1 to a subset of its target genes and to inhibit leukemia cell proliferation and self-renewal. The effectiveness of these molecules in NPM1c AML prompts the question whether NPM1c and the wildtype MLL complex cooperate on chromatin. We now show that a small fraction of mutant NPM1c is localized to the nucleus of leukemia cells where it regulates oncogenic transcription directly on chromatin.

Minimal invasive detection of circulating biomarkers in pediatric brain tumor patients using liquid biopsy

Nike Simon[1,2,3], Maaß KK[1,2,3], Fischer TT[1,2,3], Puranachot P[4], Giraud G[5,6,7], Fritzberg A[7], Öber A[5,7], Brors B[4], Pfister SM[1,2,3], Pajtler KW[1,2,3]

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Brain tumors (BT) are the most common solid tumors in pediatric patients. Invasive diagnostics pose a risk and timely detection of progression remains a major challenge. Liquid biopsies (LBs) are minimally invasive. In BTs plasma LBs are limited by the blood-brain-barrier and in cerebrospinal fluid (CSF) by low volumes. Application of improved LB methodology shows feasibility of tumor classification and early detection. Cell-free DNA (cfDNA) was isolated from CSF and plasma samples and subjected to low-coverage whole-genome-sequencing (lcWGS). Methylation status of cfDNA from CSF was assessed by EPIC array and WGS. Bioinformatic algorithms were adapted to LB specifics. LcWGS recapitulate tumor-specific copy number variants (CNVs) in CSF and plasma samples. Tumor fraction and detection rate of CNVs were significantly higher in CSF compared to plasma samples. CfDNA methylation analysis allowed for tumor classification from CSF. Clinical applicability of LB diagnostics was showcased in a patient (score 0.99) who was not amenable to a surgical biopsy. Non-invasive LBs from CSF and plasma may contribute to earlier tumor detection and timely therapy adjustments in pediatric BT patients.

Oncogenic mechanisms of fusion proteins in pediatric acute myeloid leukemia

Selina Tröster[1], Terlecki-Zaniewicz S[1], Fernandez-Pernas P[1], Eder T[1], Humer T[1], Schmöllerl J[2], Manhart G[1], Zuber J[2], Tomazou E[3], Grebien F[1]

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Oncogenic Nucleoporin 98 (NUP98) fusion proteins are found in pediatric AML with poor prognosis, but the molecular mechanisms of NUP98-fusion-driven leukemogenesis are unclear. We found that NUP98-fusion proteins form biomolecular condensates in AML cells. These chromatin-associated structures contain essential transcriptional activators and their formation is critical for the induction of oncogenic gene expression programs. Using a model for ligand-induced degradation of NUP98::KDM5A, we characterized the epigenetic and transcriptional programs that underlie NUP98::KDM5A-driven AML. CUT&Tag, nascent mRNA sequencing and data from a genome-wide CRISPR/Cas9 screen revealed direct transcriptional target genes of NUP98::KDM5A that are essential for AML cell proliferation. Among these, we validated CDK12 as a druggable vulnerability in cell lines and primary samples of NUP98::KDM5A AML. our current work focuses on mechanistic studies of the role of CDK12 in NUP98-fusion driven oncogenesis.

THURSDAY, JUNE 1st

09.00 – 10.30 **Translation and therapy I** Chair: Conny Eckert

A novel combinatorial therapy improves the outcome of t(4;11) infant pro-B-ALL through the precise induction of HDAC7 biomarker

Oriol de Barrios[1], Gusi-Vives M[1], Collazo O[1], Meler A[1], Romecin PA[2], Marschalek R[3], Bueno C[2], Stam RW[4], Menéndez P[2], Parra M[1]

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Infants younger than one year diagnosed of pro-B acute lymphoblastic leukemia (pro-B-ALL) and t(4;11) chromosomal rearrangement represent a subgroup of patients with adverse outcome, mainly due to their poor response to standard therapy. Our research has shown that expression of B-cell factor HDAC7 doubles their survival. Therefore, unveiling the mechanisms responsible for HDAC7 underexpression is essential to develop novel therapeutic strategies to improve their outcome. In this sense, we have identified a promising combinatorial therapy that precisely triggers HDAC7 in t(4;11) pro-B-ALL. This treatment promotes a whole transcriptomic reprogramming, driving leukemic pro-B cells towards a more differentiated and less malignant B-cell state and altering pathways such as cell proliferation and chromatin remodelling. After *in vitro* validation, we have obtained promising results *in vivo*, demonstrating that it reduces leukemogenesis of primary infant t(4;11) pro-B-ALL cells in mice models. Since infants are normally excluded from clinical trials due to short age and vulnerability, this HDAC7-inducing therapy opens a new field in research for personalized treatments in infant leukemia.

CRISPR/Cas9 gene therapy approaches for congenital neutropenia

Malte Ulrich Ritter[1], Masoud Nasri[1], Cornelia Zeidler[2], Karl Welte[1], Julia Skokowa[1]

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The bone marrow failure syndrome congenital neutropenia (CN) is characterized by low peripheral blood neutrophil count (<500 / µl). Although most CN patients respond to daily treatment with subcutaneous injections with rhG-CSF, some patients do not respond to this cytokine at doses up to 50 µg/kg/day. Some patients continue suffering from frequent infections despite of G-CSF therapy, while in others, especially in puberty or adult ages, G-CSF causes side effects leading to treatment discontinuation and placing them at a high risk of developing fulminant severe infections8. The only curative treatment for CN is currently the allogeneic hematopoietic stem cell transplantation which still has a 17 % 5-year mortality rate and severe side effects in 27 % of patients. There is an unmet need for an alternative safer curative therapy for CN patients. We have developed several CRISPR/Cas9-based gene therapy strategies for CN patients harboring ELANE or HAX1 mutations. We have compared the efficacy and safety profiles of these strategies in primary hematopoietic stem and progenitor cells (HSPCs) of CN patients as well as in vivo engraftment potential of gene-edited HSPCs. We are in the process of setting up the criteria for patients` and constructs` selection for clinical gene therapy trials. These critical aspects will be presented and discussed during the presentation of the data.

Restoration of miRNA-193b-3p function is a potent therapeutic option in acute myeloid leukemias

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A great obstacle in treating acute myeloid leukemia (AML) is managing toxicity, particularly in terms of bone marrow regeneration and the restoration of normal hematopoiesis. Thus, a key objective of AML research is to develop novel molecualry treatment approaches to effectively eradicate leukemia and reduce the harm to healthy hematopoietic stem/progenitor cells. We found previously that the tumor suppressor miR-193b is a strong independent prognostic marker in AML and its lower expression is associated with poor clinical outcomes. In contrast, miR-193b is upregulated in normal hematopoietic stem cells and hence represses the down-stream MAPK/ERK cascade. Here we ultiluzed synthetic mimics encapsulated into lipid nanoparticles to restore miR-193b functions in various AML patient-derived xenografts. Using comprehensive assays in vivo, we demonstrate that restoring miR-193b functions is safe and effective in restricting leukemia progression. These results provide promising evidence for miR-193b-based interventions in AML.

MENIN INHIBITION IN HOX/MEIS1 DYSREGULATED AMLS

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Princess Maxima Center for pediatric Oncology, Utrecht, The Netherlands.

Acute myeloid leukemia (AML) has still a comparably poor outcome in children. NUP98-r, MLL-r, UBTF-TD and NPM1-mutant AML subsets cluster together based on a shared transcriptional program. The observed therapeutic efficacy of Menin inhibitors in MLL-r and NPM1-mut AMLs raises the question of whether other leukemias with similar transcriptional patterns are equally responsive. Methods: Utilizing multiple techniques, including cell proliferation assay, colony formation assay, RNA-seq, proximity ligation assay, CUT&RUN, and flow cytometry, we assessed Menin inhibitor sensitivity in patient samples with dysregulated MEIS1/HOX expression. Results: Menin inhibition impaired proliferation of NUP98r, UBTF-TD and NK AMLS with similar efficiencies as observed for MLL-r AMLs. This is accompanied by suppressed colony formation, reduction of epigenetic activation marks such as H3K4me3, and global gene expression changes such as MEIS1, FLT3, IGF2BP2, and PBX3. Summary: Menin-MLL interaction plays a crucial role in a wide range of primary AMLs suggesting that these patients will benefit from inclusion into clinical evaluation of Menin inhibitors.

Identifying Gene Targets for Drug repurposing to prevent Cancer in children with a premalignant state

Anna-Lena Schmell[1,2,3], Meier K[4], Alejo O[1,2,3], Heckl D[4], Bhayadia R[1,2,3], Klusmann JH[1,2,3]

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Children with Down syndrome have a 30% risk of developing a condition called transient abnormal myelopoiesis (TAM) during prenatal development. TAM is a preleukemic disorder that can progress to myeloid leukemia (ML-DS). Progression prevention from TAM to ML-DS was so far unsuccessful, while ML-DS treatment options are limited by the availability of clinical trials for Down syndrome patients. As groundwork for overcoming these issues, we use hematopoietic cells at appropriate development to put them into an aggressive TAM-like state. We used this model in combination with CRISPR-Cas9 technology to screen genes associated with FDA approved therapeutics. Results were incorporated with screening data from a ML-DS a cell line, therefore leading to the identification of potential gene targets for

TAM and ML-DS. The potential of this approach is not only the identification of gene targets but also the discovery of associated therapeutics. In vitro testing has yielded promising results with the potential to add to the limited ML-DS treatment repertoire and to contribute to the long-term goal of preventing cancer in children with a premalignant state.

Characterizing the role of BCL-2 family members as targets for anti-cancer therapy in T-ALL

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In T-ALL deregulated cell death pathways contribute to leukemogenesis and therapy failure. Apoptosis is controlled at the mitochondria by pro- and anti-apoptotic regulators. The anti-apoptotic members of the BCL-2 family are often upregulated in ALL and thus appear as targets for therapy. Protein levels of BCL-2 family members and complexes of pro- and anti-apoptotic proteins were determined by western blot and immunoprecipitation. Cell viability assays were performed upon exposure to the BH3-mimetics venetoclax, A-1331852, S63845, AZD4230 and AZD5991. Dependencies on apoptotic regulators of T-ALL cells were assessed using BH3-profiling. We found that T-ALL cells are mostly sensitive to BCL-XL inhibition and resistant to BCL-2 inhibition. The dual BCL-2/BCL-XL inhibitor AZD4230 showed high effectivity with EC50-values in the nanomolar range in most samples. Analyses of protein complexes demonstrated on-target activity. Synergism was found upon combined inhibition. BH3-profiling was able to predict these combination effects. We demonstrated, that the dual BCL-2/BCL-XL inhibitor AZD4320 shows strong anti T-ALL activity and synergism with co-inhibition of MCL-1.

11.00 – 12.30 **Signal transduction** Chair: Pablo Menendez

Exopolyphosphatase Activity of PRUNE Regulates Adaptive Starvation Responses in Cancer Cells

Büsra Cinar[1], von Pappenheim FR[2], Schreek S[1], Khalida Ibrahim N[1], Loxha L[1], Niessen J[1], Stasche AS[1], Stanulla M[1], Tittmann K[2], Hinze L[1]

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Tolerance of amino acid starvation is crucial for cancer cells to promote cell fitness upon nutrient deprivation. In response to starvation of asparagine, leucine, and valine, GSK3α - a key regulator of the amino acid starvation response - undergoes supramolecular assembly. This response triggers efficient protein degradation as an alternative nutrient supply by concentrating GSK3α with the ubiquitin-proteasome system into cytoplasmic GSK3α bodies. However, the mechanistic underpinnings of how cancer cells can regulate GSK3α assembly have yet to be understood. We here identified the protein PRUNE, the only known mammalian exopolyphosphatase (PPX), as a novel regulator in this adaptive cellular response. PRUNE selectively binds to GSK3α in the absence of asparagine, leucine and valine. Notably, loss of PRUNE completely impairs formation of GSK3α bodies, and highly sensitizes resistant cancer cells to amino acid starvation in dependence of its PPX activity. Thus, for the first time we here identify polyphosphate levels as a fundamental regulator of the response to amino acid shortage in leukemia cells, opening an avenue for therapeutic intervention.

Leukaemia in the CNS niche regulates PI3K/Akt signalling via upregulation of miR-93 in MLL-AF4+ infant leukaemia

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One of the unique clinical features of infant leukaemia is a high rate of central nervous system (CNS) disease, typically a leukaemic infiltrate of the meninges. Using a fully murine MLL-AF4+ infant leukaemia model (Malouf et al. Blood 2021), which develops this characteristic leukaemic infiltrate of the meninges, we performed RNA sequencing to identify niche-specific drivers of CNS leukaemia. Differential gene expression analysis of BM and CNS-derived leukaemia identified genes involved in PI3K/Akt signalling pathway specifically Pten and Cdkn1a. We confirmed these findings on a protein level and showed CNS-derived leukaemia cells have increased activation of this pathway. In silico mRNA-miRNA analysis identified the miR-17-92 family as potential regulators of this interaction. We profiled the miR-106b-25 cluster within this family and found upregulation of miR-93 in CNS-derived leukaemia cells. Subsequent analysis of MLL-AF4+ infant leukaemia patient-derived xenografts have mirrored the miR-93 expression pattern found in our model. Further work is underway to understand the niche-specific functional importance of miR-93 and its potential as a biomarker for CNS leukaemia.

Medulloblastoma EVs influence TGF-beta/SMAD1 signaling in mesenchymal stem cell

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Increased TGF-β expression and extracellular TGF-β signaling provides either tumor with pro or anti-survival benefits in the tumor microenvironment by immune evasion of the cancer cells. In the present study, we evaluated the role of medulloblastoma extracellular vesicles (MB-EV) on influencing TGF-β signaling on bone marrow derived mesenchymal stromal cells (BM-

MSC). EV isolated from conditioned medium of DAOY and ONS76 cell lines decreases mesenchymal stem cells differentiation by blocking the TGF-beta downstream targets and migratory molecules. In addition, we observe high expression of TGF-beta like protein I (TGFBI) on the EVs isolated from MB cell lines and patient blood plasma from suggesting the potential role of this protein in impairing TGFbeta/SMAD1 signaling pathway leading to functional changes on the BM-MSC. In summary, we conclude that EV associated TGF beta in medulloblastoma influences BM-MSC by influencing of TGFbeta/SMAD1 axis.

Effective targeting of Wnt Signaling in B Cell Precursor Acute Lymphoblastic Leukemia

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Acute lymphoblastic leukemia (ALL) is the most frequent malignant disorder in children and adolescents with the majority deriving from B-cell precursor lineage (BCP). Dysregulated Wnt signaling is often found in malignant hematopoiesis and contributes to leukemogenesis. We analyzed gene expression profiles of Wnt signaling in BCP-ALL patient-derived xenograft samples and found CTNNB1, CCND1/2 and LEF1 gene expression to be significantly increased compared to remission controls, pointing to aberrantly activated Wnt signaling in BCP-ALL. Next, we used a library of small molecule Wnt inhibitors and identified two inhibitors which effectively induced leukemia cell death along with a profound reduction of β-catenin (western blot), suggesting abrogation of Wnt signaling in BCP-ALL upon Wnt inhibitor treatment. In conclusion, aberrantly increased Wnt gene expression was identified in BCP-ALL that could be effectively targeted by Wnt inhibitors highlighting the implication of pre-clinical application in BCP-ALL.

Wnt/STOP activation drives temporally dynamic ribosomal biogenesis in drug resistant leukemia cells

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Studies have demonstrated that resistant leukemia cells depend on GSK3α-mediated proteasomal degradation as an alternative source of amino acids to survive treatment with asparaginase. Inhibition of this degradation machinery, termed Wnt-dependent stabilization of proteins (Wnt/STOP), limits generation of free amino acids, creating a therapeutic vulnerability. However, the underlying signaling processes bridging the activation of Wnt/STOP and cell death remain elusive. Here, we show that unexpectedly Wnt/STOP mediated apoptosis is independent of known stress signaling pathways such as the unfolded protein response or the kinase GCN2 whose main characteristic is the sensing of amino acid depletion within the integrated stress response. Additionally, we could find independence from changes in cell cycle progression and expression of the asparagine synthesizing enzyme ASNS. Instead, leveraging RNA-sequencing we could identify a temporally dynamic role for ribosomal proteins in mediating Wnt/STOP induced apoptosis. Collectively, our findings indicate ribosomal biogenesis as a previously unrecognized key factor in Wnt/STOP mediated asparaginase sensitization.

RAS pathway mutations drive oncofetal reprogramming in hematopoietic stem cells

Maximilian Schönung, Hartmann M, Lipka DB

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Juvenile myelomonocytic leukemia (JMML) is a pediatric myeloproliferative neoplasm caused by mutations of the RAS signaling pathway. We recently established DNA methylation

epitypes as a prognostic biomarker in JMML. Yet, the functional role of aberrant DNA methylation and its implication in JMML pathogenesis remained elusive. We conducted a multi-modal analysis to investigate the molecular alterations associated with the JMML epitypes. Hematopoietic stem cells (HSCs) from JMML patients revealed fetal-like gene expression and DNA methylation patterns, suggesting a reprogramming of these cells to an oncofetal state. This effect was most prominent in JMML patients assigned to the high methylation epitype. To experimentally verify that RAS pathway mutations can induce oncofetal reprogramming, we established a JMML mouse model that relies on the induction of Ptpn11-E76K mutations in HSCs of juvenile mice. HSCs from Ptpn11-E76K mutant mice exhibited myeloid priming and activation of fetal-like gene expression programs mimicking the signatures observed in human JMML. In conclusion, we demonstrate that RAS pathway mutations are sufficient to induce oncofetal reprogramming in JMML HSCs.

Association and interactions of the RIO kinases in the context of Diamond-Blackfan anemia

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Diamond-Blackfan anemia (DBA) is a rare and phenotypically variable inherited bone marrow failure syndrome caused by mutations in RPS and RPL genes and thus generally considered a ribosomopathy. Main symptome is a maturation block during erythropoiesis, a process tightly regulated by transcription factors such as GATA1. We recently identified a RIOK1 duplication in a DBA patient who did not have a RPS/RPL mutation. RIOK (right open frame kinase) proteins (i.e. RIOK1, RIOK2, RIOK3) play a plethora of roles, especially in the maturation of the 40S subunit of ribosomes. RIOK2 was recently described to play a major role in haematopoiesis by affecting transcription factors like GATA1/2, SPI1, RUNX3 and KLF1. We are investigating whether also RIOK1 is involved in haematopoiesis. We showed that RIOK1 overexpression resulted in a rapid loss of hematopoietic stem and progenitor cells and a reduced propensity to differentiate towards red blood cells. We conclude that RIOK1 is a novel DBA-associated gene and are currently investigating whether and how RIOK1 and RIOK2 cooperate during hematopoiesis.

14.00 – 15.30 **Immunotherapy I** Chair: Lüder Meyer

A novel fratricide-resistant CAR-T cell immunotherapy for T-ALL

Néstor Tirado, Martínez-Moreno A, Fernández-Fuentes N, Díaz VM, Vinyoles M, Bueno C. Menéndez P, Sánchez-Martínez D

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Chimeric antigen receptor (CAR)-T cell immunotherapies have revolutionized the treatment of B cell malignancies, in contrast to T cell acute lymphoblastic leukemia (T-ALL) where they are still lacking. The main obstacle to the development of CAR-T therapies in T-ALL is the shared expression of target antigens between leukemic and healthy T cells, leading to CAR-T fratricide and life-threatening T cell aplasia. We previously developed a CD1a-directed CAR-T strategy for the treatment of cortical T-ALL, a major subtype amounting to 40% of all T-ALL cases, that circumvents these limitations and is now part of a phase I clinical trial (NCT05679895). Here, we report a new CAR-T immunotherapy of T-ALL against a novel target antigen. We validated our antigen's specificity and safety profile in a large cohort of healthy and leukemic primary samples. We generated a proprietary IgG hybridoma and cloned the humanized sequence of the antigen-binding fragment into a CAR backbone. Our transduced CAR-T cells efficiently and specifically eliminated target cells in both in vitro and in vivo models. We propose a dual immunotherapy, along with CD1a, to cover and treat over 70% of all T-ALL patients.

Inhibition of the "don't eat me signal" CD47 to prevent relapse in juvenile myelomonocytic leukemia

Jun Wang[1,2], Rajak J[1,2,3], Koleci N[1,2], Xiao H[1,2], Wehner NA[1], Orth JF[1], Niemeyer CM[1], Bohler S[1], Erlacher M[1]

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JMML is a highly aggressive childhood leukemia. The only curative treatment is allogeneic hematopoietic stem cell transplantation (HSCT) but the risk of relapse is high, especially for PTPN11 mutated patients. JMML cells express various immune checkpoints that may mediate immune escape. Amongst others, we identified the 'don't eat me' signal CD47 that prevents leukemia cell phagocytosis. We aim to understand the impact of CD47 on immune escape and to test how CD47 inhibition affects disease presentation and risk of relapse. We are using PTPN11-knockin mice, primary JMML cells and patient-derived xenograft mice (PDX). CD47 is highly expressed on human JMML and murine stem and myeloid splenic cells. Phagocytosis assays revealed that anti-CD47 could enhance phagocytosis of both, human and murine cells. Use of Magrolimab in PDX mice resulted in strong depletion of human JMML cells in the spleen and liver but only with mild depletion in the bone marrow. We will next establish a relapse JMML mouse model to test the effect of anti-CD47 treatment on relapse risk. Our studies will pave the way for future anti-CD47 antibody clinical trials.

Comparison of armored NK cells equipped with soluble and membrane-bound IL-15 variants for improved functionality

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In recent years, NK cells have gained increasing interest as a promising cell source for CAR-based immunotherapies. Despite the effective anti-tumor activity and favorable safety profile of CAR-NK cells in preclinical studies, CAR-NK cell therapy faces considerable challenges such as limited in vivo persistence restricting its clinical application. To overcome this limitation and further improve CAR-NK cell functionality, various strategies have been developed including armoring of CAR-NK cells with cytokines and cytokine receptors. To this end, we engineered primary NK cells by lentiviral transduction to express different IL-15 variants, including (i) soluble IL-15, (ii) IL-15 linked to membrane-bound IL 15Rα, and (iii) mutant (N27D) IL-15 super agonist linked to an IL-15Rα sushi domain-IgG-Fc fusion protein (ALT-803). Aiming to identify the most suitable IL-15 variant for CAR-NK cell therapy, we directly compared the influence of these IL-15 variants regarding NK cell proliferation capacity and functionality. In summary, the evaluation of the tested IL-15 variants provides the basis to further improve CAR-NK cell therapy towards enhanced persistence and in vivo functionality.

CD19-Targeted immunotherapies for Treatment of Pediatric t(8;21) Leukemia

Farnaz Barneh[1], Meulendijks T[1], Wijnen N[1], Kodijk J[1], Ashtiani M[1], Heidenreich-Krippner A[1], Dunnebach E[1,2], Nierkens S[1,2], Kaspers G[1], Heidenreich O[1,3]

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Emergence of bispecific antibodies or CAR-T cells targeted against CD19 has significantly enhanced survival of patients with B-cell acute lymphoid leukemia. However, identifying a suitable target antigen for immunotherapy in acute myeloid leukemia (AML) has been challenging. Among different subtypes of AML, t(8;21) translocations have been associated with varying degrees of CD19 expression. This raises the question whether CD19 based immunotherapies can be repurposed for this subtype of AML. Therefore, we assessed the incidence of CD19 expression in pediatric patients in the Dutch NOPHO-DBH AML-2012 cohort, and the sensitivity of CD19-expressing AML cells towards blinatumomab, a CD19xCD3 bispecific antibody, or CD19-directed CAR-Ts. Eighteen out of 167 AML patients expressed CD19 of which 11 carried the t(8;21) translocation and expressed CD19 both on immature and mature sub-populations,. Autologous killing assays with patient derived T-cells or CD19-directed CAR-Ts showed substantial sensitivity of t(8;21) AML towards T-cell mediated cytotoxicity. Our results therefore support clinical evaluation of blinatumomab and CAR-T 19 efficacy in pediatric patients with t(8;21) AML.

Radiosensitivity in pediatric solid tumors

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Pediatric solid tumors are very heterogeneous and radiotherapy is an important pillar of treatment regimes for these tumors. Radiosensitivity varies greatly between and within different entities, however this heterogeneity is not reflected in current treatment protocols. Though there is a great need for more individualized radiotherapy in pediatric oncology, research toward a molecular stratification of patients has been limited. Our comprehensive in vitro screen of radiosensitivity in 10 different tumor entities (10 cell models each) showed great heterogeneity between and within entities. Cells were irradiated with doses from 0 to 12 Gray of photon radiation. After an ATP-based survival readout the data was processed using the iTReX algorithm adapted for radiosensitivity screening. Five different parameters describing the dose-response curve were condensed into one value called radiosensitivity score. We plan to use this unprecedented experimental data set to derive a predictive gene expression

signature based on RNA sequencing data collected for each model. The aim is to utilize this biomarker as a clinical decision-making tool to improve patient stratification for radiotherapy.

Single cell multiomic analysis of clonal and transcriptional programs of CD19 CAR T cells in the immunotherapy response

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CD19-directed CAR T-cell therapy has shown high rates of complete response against relapsed/refractory B-ALL, but it is only maintained in 50% of patients after a year. The impact of CAR T-cells' phenotypic, clonal, and functional heterogeneity on clinical outcomes remains unclear. Thus, a deeper examination of how clonal kinetics and diversity of CAR T-cells translate into short-term effectiveness and long-term persistence is crucial to pinpoint. scTCR-seq and scRNA-seq were used to analyze samples from manufactured Infusion Product (IP) and peripheral blood during the CAR T-cell expansion peak (Peak) of five B-ALL patients. Our study revealed that patients with higher CD4 T-cell proportions at IP had a larger response, while patients with higher exhaustion scores had worse prognosis regardless of the presence of the CAR. At Peak, a significant increase in clonally expanding CD8+ T-cells was observed but impressive expansion of cytotoxic γδ T-cells correlated with patient outcome pointing out its importance. These findings provide insight into the interplay between the immune response and CAR-T-cell therapy and could contribute to the development of more effective treatments.

Enhancing TRAIL-mediated killing of ErbB2-CAR-NK-92 (NK-92/5.28.z) with bortezomib

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Treatment resistance of metastatic rhabdomyosarcoma (RMS) making the urgent medical need for this entity to a key issue in pediatric oncology. Immune-editing and the immune-suppressive microenvironment of solid tumors hamper chimeric antigen receptor (CAR) immunotherapies. This escape mechanism of metastatic RMS may be reversed by combining CAR-NK-92 cells (NK-92/5.28.z) with additional anticancer therapies. In this first assessment of its kind, the proteasome inhibitor bortezomib induced apoptosis in RMS cells. Furthermore, the surface expression of tumor necrosis factor related apoptosis inducing ligand (TRAIL) receptor DR5 was enhanced on RMS cells upon bortezomib treatment in a dose dependent manner. Combinational administration of bortezomib and NK-92/5.28.z showed an increased antitumor activity compared to single treatment. Cytotoxic assessment with purified TRAIL, to exclude other cell-mediated effects revealed synergism between bortezomib and TRAIL-mediated cytotoxicity in RMS cells. Thus, the combination of NK-92/5.28.z cell immunotherapy with bortezomib might be a powerful immunotherapy approach, that can be harnessed for clinical translation.

16.00 – 18.00 **Molecular mechanisms of disease I** Chair: Owen Williams

Expression of retinoic acid receptor gamma is modulated by miR-30a

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All-trans-retinoic acid (ATRA) plays critical regulatory roles in normal haematopoiesis and the pathogenesis of adult and pediatric acute myeloid leukemia (AML). While ATRA can inhibit growth and stimulates myeloid differentiation via RARα, it is equally potent in causing expansion of haematopoietic stem cells via RARγ. RARG mRNA is expressed in AML patients and normal stem/progenitor cells but not in more mature myeloid cells. Changes in RARγ expression are paralleled by a reciprocal change in expression of RARG 3'-targeting miRNAs (mIR-24, mIR-30a, mIR-331). RARγ protein and RARG mRNA are also expressed in cell lines derived from primary AML samples but not in ATRA-responsive AML cell lines. Lastly, expression of mIR-30a in TEX cells promotes differentiation and inhibits proliferation. Our results suggest that miRNA-mediated down-regulation of RARγ expression in the myeloid lineage switches ATRA responsiveness from RARγ-mediated pro-proliferation to RARα-mediated pro-differentiation and that the combinatorial use of RARα and RARγ selective agonists and antagonists, respectively, could be effective in retinoid-based differentiation therapy of AML.

Impact of posttranscriptional regulations of MLL-AF4 and its target Genes in pro B-ALL

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The chromosomal translocation t(4;11) results in the expression of MLL-AF4 and AF4-MLL, and is associated with the onset of pro B-ALL in infant, children and adults. Although this particular translocation is the most frequent *MLL*-rearrangement, the precise cancer mechanism is still unclear, and a satisfactory *in vivo* model for pro B-ALL only exists in CRISPR/Cas9 model systems. Given the fact that MLL-AF4 alone is unable to transform hematopoietic cells, other possibilities have been discussed, e.g. specific cells-of-origin need for cellular transformation, or that the reciprocal fusion is the true driver of leukemogenesis. Experimental evidence is pointing to another mechanism, namely the impact of post-transcriptional mechanisms via RNA-binding proteins (RBPs) for the abundance of the MLL-AF4 fusion transcript. Here, we present first data on RBPs and potential binding motifs which differ in mouse and human AF4 sequences. As a consequence, human MLL-AF4 could only be expressed at low levels, because otherwise this transcript is too toxic for mammalian cells.

The role of the DACH family of nuclear proteins in MLL-AF4-associated infant leukaemia

Hélène Jakobczyk, Ottersbach K

Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK.

The most common type of infant leukaemia is caused by the t(4;11) translocation that fuses the MLL and AF4 genes. RNA-Seq studies have recently been completed in the lab to gain a more comprehensive insight of this disease. Two genes from the same family of nuclear proteins presented opposite expression patterns in patient samples. A lack of expression of the DACH1 gene, which has been described as a tumour suppressor in a range of different cancers, was noted. At the same time, an overexpression of the SKIDA1 gene, which appears to be an oncogene, was observed. The aim of this work is to assess the impact of DACH1 and

SKIDA1 on the initiation of MLL-AF4+ infant leukaemia and on its maintenance. In our pre-leukaemia mouse model and in the SEM leukaemia cell line, we observed that DACH1 has a general negative effect on the survival of the cells and on their differentiation whereas SKIDA1 has the opposite effect. These data give new insights into the role of DACH1 and SKIDA1 in MLL-AF4 leukaemia. In the future, we will identify downstream targets of these proteins to determine which genes are directly regulated by them to counteract the leukaemic phenotype.

Deciphering the role of KANSL1 mutations in the development of Myeloid Leukemia in children with Down Syndrome (ML-DS)

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Transient abnormal myelopoiesis (TAM) is a form of clonal hematopoiesis seen in infants with trisomy 21. TAM is caused by mutations in the transcription factor GATA1, leading to the expression of a shortened isoform (GATA1s). TAM clonally evolves at high percentage to myeloid leukemia in Down syndrome (ML-DS) upon acquisition of secondary mutations. Leveraging a virus-free CRISPR platform to introduce GATA1s and additional mutations in primary human fetal liver hematopoietic stem and progenitor cells (hFL-HSPCs) followed by in vitro testing and in vivo xenotransplantation assays, we revealed KANSL1 loss to be a potent oncogenic event driving progression from clonal hematopoiesis to frank leukemia. To define the role of KANSL1 mutations in normal and malignant hematopoiesis, we are performing loss-of-function assays to investigate KANSL1 essentiality. Furthermore, we are establishing dTAG degron knock-in cell lines for proteomic, transcriptomic and epigenomic assays in order to define the KANSL1 interactome in leukemic cells. Future research will explore potential new therapeutic vulnerabilities in ML-DS patients carrying mutated KANSL1.

Examination of RNA exosome mediated RNA decay as context-dependent cellular vulnerability

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Since its first application in the first half of the 20th century, chemotherapy has revolutionized and still determines treatment concepts of many malignant diseases. Exploiting the cancerous cell's increased replication rate, classic chemotherapeutic regimens are often guided by the patient's treatment response, not primarily accounting for the individual malignant genotype. In contrast, usage of "small molecules" in "targeted" therapy relies on identification of patient-specific so called "cancer vulnerabilities". Such vulnerabilities can arise from situations, in which a combined phenotype of two gene perturbations has a more severe fitness defect than would be expected from single gene deficiency, referred to as negative genetic interaction (GI) or synthetic sickness. A typical example is the clinically exploited synthetic sickness between BRCA and PARP. In order to identify new promising cellular vulnerabilities, pooled CRISPR-Cas screens offer a powerful means to interrogate vast amounts of genetic perturbations for GIs and functional phenotypes in different model organisms – from cell culture to direct *in vivo* delivery. After identifying involvement of several RNA metabolic pathways in cancer, such as distinct splicing programs and altered expression of RNA decay factors, we wonder whether similar context dependent differences hold true for adaptor complexes of the RNA exosome, the main nuclear 3'->5' ribonuclease machinery, parts of which have only recently been

identified. To this end we apply and develop CRISPR screening tools, including gene perturbation libraries designed to target different RNA metabolic pathways in several glioblastoma cell lines.

UNDERSTANDING NFE2L2/KEAP1-MEDIATED DRUG RESISTANCE IN HEPATOBLASTOMA

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Hepatoblastoma (HB) is the most common type of liver cancer in children and accounts for less than 1% of all pediatric tumors. Despite improvements in the clinical management of HB patients over the last decades, there is still a significant portion of patients that face poor outcome. Mutations in the NFE2L2 or KEAP1 gene occur in approx. 5% of HB patients and have been associated with poor response to standard chemotherapy. In this study, we used CRISPR-Cas9 technology to create NFE2L2- and KEAP1-activated liver cancer models. The newly established NFE2L2- or KEAP1-mutated clones showed an increased NFE2L2 activity, increased cell growth and lower sensitivity towards cisplatin and doxorubicin treatment compared to the parental cell lines. RNA sequencing and integration of transcriptomic data into the drug prediction tool DrugSense allowed us to identify several potential drugs selectively targeting NFE2L2/KEAP1-activated liver cancer cells. Our study provides first insights into the molecular biology of NFE2L2 activated pediatric liver cancers and may lead to the development of novel treatment strategies for these patients.

TRIP13 AS A POTENTIAL THERAPEUTIC TARGET IN NUP98-JARID1A PEDIATRIC NON-DS-AMKL

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The chromosomal rearrangement involving NUP98 and KDM5A/JARID1A (NJ) accounts for approximately 15% of pediatric non-Down Syndrome Acute Megakaryoblastic Leukemia (non-DS-AMKL) cases and correlates with poor prognosis. In this study, we demonstrate that the fetal transcriptional landscape creates a conducive environment for malignant transformation by NJ, which can be therapeutically targeted.

In vivo experiments revealed that NJ expression in murine hematopoietic stem/progenitor cells originating from fetal liver (FL) and bone marrow (LSK) displayed a more aggressive phenotype in the fetal cell background. Suspecting a connection between fetal gene programs and NJ-mediated transformation, we performed a CRISPR-Cas9 screen targeting fetal signatures. By comparing our findings with two other FL leukemia models, we identified TRIP13 as a high-confidence candidate gene with exclusive dependency in NJ FLCs.

At the molecular level, TRIP13 inhibition disrupts cell proliferation in a TP53-dependent manner. We capitalized on this discovery by employing combined pharmacological inhibition of TRIP13 and MDM2. In summary, our study uncovers TRIP13 sensitivity in NJ-driven leukemia and offers valuable insights into potential therapeutic strategies for the treatment of high-risk pediatric AMKL.

Identification and functional characterization of CTCF-bound noncoding RNA loci in acute myeloid leukemia.

Marit Vermunt, Klusmann JH

Goethe-University Frankfurt, Frankfurt/Main, Germany

Epigenetic modifiers are frequently mutated in cancer. DNA methylation enzymes as well as architectural proteins such as CTCF can be affected, and a number of therapies target the epigenome to reverse aberrant gene expression. Here, we will focus on noncoding RNA loci bound by CTCF (C-LNCs) in acute myeloid leukemia (AML). We have recently discovered that CTCF blocks noncoding antisense transcription at hundreds of bidirectionally transcribed promoters. CTCF binding, on the other hand, is blocked by DNA methylation. To study how these epigenetic features contribute to AML, we will identify C-LNCs in leukemic cells and determine whether they are tumor dependencies via CRISPR screens. In addition, we will investigate whether cancer cell-specific noncoding RNAs can be used as biomarkers, and potentially even targets for immunotherapy if they are translated into microproteins. Lastly, we will explore how drugs targeting the epigenome affect the processes described above. In sum, these experiments will increase our understanding of how epigenetic changes contribute to AML and how these alterations can be used to identify and/or target tumor cells specifically.

FRIDAY, JUNE 2nd

09.00 – 10.30 **Immunotherapy II** Chair: Irmela Jeremias

Blocking TIM3:Galectin-9 pathway enhances in vivo CAR19T cell function

Aïda Falgàs[1], Zanetti SR[1], Martínez Moreno A[1], Panisello C[1], Romecin PA[1], Díez Alonso L[2], Álvarez Vallina L[2], Bueno C[1], Menéndez P[1]

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One-year relapse rates of leukemia patients treated with CD19-targeted CAR-T cells (CAR19T) are >60% partly due to CAR19T intrinsic mechanisms and their interaction with leukemic cells and their microenvironment. Here, we have comprehensively characterized the expression of inhibitory immune checkpoint receptors (ICRs) in T-cells, and their ligands in both leukemic cells and mesenchymal stromal cells (MSC) from bone marrow (BM) of pediatric and adult primary B-cell acute lymphoblastic leukemia (ALL) patients at diagnosis and relapse. Among all the ICRs-ligands analyzed, our results reveal a significant upregulation of the ICR TIM3 and its ligand Galectin-9 in T-cells and B-ALL/MSCs, respectively, during disease progression. The expression of TIM3 and Galectin-9 was significantly upregulated by CAR19Ts and CAR19T-resistant B-ALL cells, respectively, after in vitro cytotoxicity assays. Further in vivo assays using a TIM3 molecular decoy engineered to be secreted by T cells underpinned an inhibitory role for TIM3:Galectin-9 axis in CAR19T cell function and expansion. Targeting TIM3:Galectin-9 axis may represent a promising co-adjuvant therapy in B-ALL patients treated with CAR19Ts.

Comparison of CD28- and 4-1BB-based CLEC12A-CAR-natural killer cells against acute myeloid leukemia

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Clonal heterogeneity and antigen escape mechanisms in acute myeloid leukemia (AML) make it difficult to find a suitable target antigen for immunotherapy against this disease. Yet, CLEC12A constitutes a promising target, as it is expressed on the majority of AML blasts, whilst being absent on healthy hematopoietic stem cells. We generated two different CLEC12A targeting CAR-NK cell products, incoporating either a CD28 or 4-1BB co-stimulatory domain. CAR-NK cells were assessed in 4h- and 24h-cytotoxicity assays against CLEC12A+ OCI-AML2 cells. CAR-NK cells showed significantly enhanced killing compared to non-transduced (NT)-NK cells, while no significant difference between the two CAR constructs was observed. CAR-NK cells were then tested in an OCI-AML2 xenograft mouse model. Mice treated with 4-1BB-based CAR-NK cells showed significantly reduced leukemic burden and absence of bone marrow-located OCI-AML2 cells compared to mice which received CD28-based CAR-NK or NT-NK cells. These results suggest CLEC12A as a promising target for the treatment of AML. Additionally, the co-stimulatory domain 4-1BB seems to have a beneficial effect on CAR-NK cell persistance and efficacy in vivo.

Spatial analysis reveals distinct immune phenotypes and tertiary lymphoid structure-like aggregates in pediatric AML

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Pediatric cancers are characterized by a relatively low mutational burden and therefore, children are thought to be poor candidates for T cell-engaging immunotherapies. Here, we performed a multidimensional characterization of the tumor immune microenvironment in newly diagnosed children with acute myeloid leukemia (AML) and non-leukemic controls. We identified a subset of pediatric AML patients with remarkably high levels of T cell infiltration and a relatively low abundance of anti-inflammatory macrophages in the bone marrow. In addition, we detected large T cell networks that colocalized with B cells in immune-infiltrated samples, resembling tertiary lymphoid structures as described in solid tumors. Using spatial transcriptomics, we dissected the composition of these structures and revealed unique hotspots of anti-tumor immunity. This work raises the possibility that a subset of pediatric AML patients may benefit from T cell-engaging immunotherapies and encourages further study of these lymphoid structures in the context of immunotherapy in AML.

Rituximab clearance in pediatric patients with mature aggressive B-cell Non-Hodgkin Lymphoma

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Background: In recent decades, treatment of pediatric patients with mature aggressive B-cell Non-Hodgkin lymphoma (B-NHL) has been systematically improved to overall survival rates of 80-90%. Current chemotherapeutic regimen in combination with B-cell targeting agents, like the anti-CD20 antibody rituximab improve the outcome for high-risk patients. To optimize rituximab efficacy in children with B-NHL by dose-adjustment we analyze potential parameters influencing the individual clearance of rituximab. Design: 144 children, enrolled in the trial B-NHL 2013, were monitored for rituximab levels at day 5 after the first rituximab infusion. Rituximab concentrations were determined with an ELISA assay established according to the method of Hampson et al., 2010. The concentrations were correlated to patient-specific parametersregistered to the NHL-BFM data center. Results and Conclusion: Rituximab levels were characterized by inter-individual variability. Besides the already reported association of rituximab clearance with tumor burden, we observed additional individual parameters, which potentially affect the clearance and might be useful for concepts of dose adjustment.

NK cell immunotherapy against chemotherapy-resistant rhabdomyosarcoma

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In children and young adults, rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma. Standard therapeutic concepts include surgery, chemotherapy and radiotherapy. Nevertheless, resistance can occur and the five-year survival rate of patients with metastatic or relapsed RMS is below 30% - underlining that new therapeutic approaches are urgently needed. Natural Killer (NK) cell immunotherapy has shown promise for the treatment of hematological diseases. Here, we investigated the interactions between NK cells and wildtype as well as chemotherapy-resistant RMS cell lines to learn more about RMS resistance and responsiveness to NK cells. We compared five parental RMS(RD, RH30, RH41, UKF-Rhb1 and RH36) to their vincristine-resistant sublines, as vincristine (VCR) is one of the standard cytostatic drugs used to treat RMS. To further improve NK cytotoxicity and allow specific retargeting, EGFR-CAR-NK cells were tested against the vincristine-resistant RMS cell lines. In addition, we plan to analyze chemotherapy-resistant Neuroblastoma cell lines, another common pediatric solid tumor.

Choosing the right effector cell for ErbB2-CAR-engineered immunotherapy in rhabdomyosarcoma – CAR-T vs CAR-CIK

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Engineering immune effector cells with chimeric antigen receptors (CAR) to specifically target cancer cells has achieved promising results. However, there are limitations especially for the treatment of solid tumors. Cytokine-induced killer (CIK) cells harboring a predominantly T-, with mixed natural killer (NK) phenotype have been used as alternative effector cells and shown potent, non-MHC-restricted cytotoxicity. In this first direct comparison of CAR-T versus CAR-CIK cells we targeted ErbB2+ rhabdomyosarcoma. Generated from PBMCs of healthy donors, T and CIK cells both showed robust CAR-expression. In vitro immunotoxicity revealed comparable efficacy of CAR-T and CAR-CIK cells against RMS. Antitumoral effects seemed to be primarily implemented by CD8+ and effector memory cells. Of note, CAR-CIK cells showed higher cytotoxicity against 3D tumor spheroids in vitro. In NSG mice xenografted with ErbB2+ human RMS tumors both cells impaired tumor development leading to a significantly improved survival. In our preclinical analyses, we clearly showed non-inferiority of novel CAR-CIK vs. conventional CAR-T cells.

11.00 – 12.30 **Translation and therapy II** Chair: Dirk Heckl

From bench to bedside – Establishing PRMT5 inhibitors in the treatment of Glioblastoma multiforme

Liana Gahleitner[1], Braun C[1,2,3]

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Curative therapy options for Glioblastoma multiforme (GBM) in children and adolescents are still sparse. With PRMT5 (protein arginine methyltransferase 5) a new target for personalized medicine has emerged. PRMT5 is involved in transcription, alternative splicing and tumorigenesis. It is frequently overexpressed in various cancer entities and a promising molecular target. Various small molecule inhibitors have been developed. Although PRMT5 inhibitors have shown promising results in clinical trials with adults, pediatric patients have not yet been treated with these new inhibitors. I compared three PRMT5 inhibitors (JnJ-64619178, PF-06939999, and GSK-3326595,) based on their potency, estimated BBB penetration, on target activity, and cellular effects in vitro using adult as well as pediatric GBM cell lines. All three inhibitors showed strong efficacy and measurable influence on the cell cycle in vitro. A known biomarker identified in adult GBM can also be used to select pediatric patients who could benefit from treatment with PRMT5 inhibitor compounds. Collectively, these results show the strong potential PRMT5 inhibitors have in the therapy of pediatric GBM.

A screening of donated chemical probes to identify novel therapeutic options for childhood AML

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Despite intensive treatment regimens, the survival rate for children with acute myeloid leukemia (AML) has not improved beyond 70%. Recently, targeted therapies and small molecules have emerged as promising alternative treatment options. To explore these options, we conducted a screening of 105 small molecules in four different AML cell lines, followed by a screening of promising compounds in patient-derived cells of childhood AML. In addition to HDAC and BET inhibition, we found that inhibition of Cdc2-like kinases (CLKs) effectively reduced cell viability in both cell lines and patient-derived cells. CLKs play a key role in splicing. Alternative splicing isassociated with solid andhematological malignancies through mutations in splicing factors and alterations in the expressionlevels of splicing regulatory factors. This gives us reason to believe that CLK inhibition may represent apromising new therapeutic option for pediatric AML.

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

Alexandra Niedermayer, Enzenmüller S, Seyfried F, Debatin K-M, Meyer LH

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Apoptosis induction is counter-regulated by anti-apoptotic molecules like BCL-2 and MCL-1; selective inhibition of BCL-2 by venetoclax (VEN) shows (pre-) clinical activity in hematological malignancies including BCP-ALL. Hyperactivated mTOR signaling is associated with inferior relapse-free patient survival in ALL. Here, we evaluated anti-leukemia activities of BCL-2 (VEN) and mTOR/PI3K pathway (NVP-BEZ235) inhibition. Both, cell lines and PDX samples

showed heterogeneous responses to VEN. High MCL-1 expression in VEN insensitive (VENins) samples pointed to MCL-1 as a mediator of VEN insensitivity. Exposure of ALL cells to NVP-BEZ235 (BEZ) resulted in decreased phosphorylation of the downstream targets S6 and 4E-BP1, reduced cellular proliferation and most interestingly downregulated MCL-1 protein expression. Co-treatment with VEN and BEZ synergistically induced cell death in BCP-ALL, including apoptosis deficient, VENins and poor outcome leukemias. Taken together, we show anti-leukemia activity of simultaneous PI3K/mTOR and BCL-2 inhibition priming apoptosis deficient, VENins leukemias to synergistic cell death induction along with downregulation of anti-apoptotic MCL-1.

Investigating and modulating leukemia initiating cells to reduce risk of post-transplant relapse in JMML

Hui Xiao[1], Koleci N[2], Wang J[3], Rajak J[1], Wehner NA[1], Bohler S[1], Orth JF[1], Kern U[1], Niemeyer CM[1], Erlacher M[1]

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JMML is a myeloid neoplasm of early childhood driven by active RAS signaling and epigenetic mechanisms. Allo-HSCT is the only curative treatment for most JMML patients. Surprisingly, relapse risk after allo-HSCT is different in the genetic JMML subtypes. In particular, PTPN11 mutated and DNA-hypermethylated JMML relapse in up to 50% cases, while KRAS mutated JMML almost never relapses. One conceivable explanation is that Ptpn11 mutated leukemia initiating cells (LICs) might have stronger competitive fitness than healthy HSCs while Kras mutated LICs might be outcompeted by donor hematopoietic stem and progenitor cells (HSPCs).We are studying LIC fitness on a functional and transcriptomic level to investigate and modulate LICs to reduce relapse risk . We are comparing bone marrow and splenic HSPCs of mice expressing the Ptpn11 D61Y/+ and KrasG12D mutants with WT cells, with focus on proliferation, self-renewal, differentiation and apoptosis. Our preliminary data show that Ptpn11 mutated LSK cells accumulate in the spleen. They proliferate more and faster when compared to WT LSK cells but are characterized by a differentiation defect.

Tunable control of G-CSFR signaling by de novo designed modulators

Timo Ullrich, Hernandez-Alvarez B, Welte K, Skokowa J, ElGamacy M

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Recent leaps in protein design technologies have enabled the bottom-up design of novel protein structures with tailored functions. These advances have lately opened new frontiers in cytokine biology, enabling the design of synthetic modulators of cytokine receptors. In this work, we use protein design to probe the structure-function relationship of the granulocyte-colony stimulating factor receptor (G-CSFR). Our approach started with the design of hyperstable G-CSFR-binding proteins. By tailoring these proteins' affinity, oligomeric state, and shape, we could create activators, inhibitors, and tuning modulators, respectively. Specifically, we designed and characterised enhanced agonists and antagonists with superior functional and biophysical properties, that are capable of potently inducing or inhibiting granulopoiesis, respectively. Moreover, we created a range of ligands with varying shapes to associate the G-CSFR subunits in non-native geometries. These geometry-rigging ligands can be used to tune the receptor activity, and subsequently, the downstream cellular response. Altogether, our results demonstrate the strong therapeutic potential of these designs to tackle different disorders of granulopoiesis.

Targeting the fetal transcriptional landscape of pediatric AML

Jessica Santosa Hidajat[1,2,3], Heckl D[4], Schmell AL[1,2,3], Bhayadia R[1,2,3], Klusmann JH[1,2,3]

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Acute myeloid leukemia (AML) is caused by an accumulation of genetic aberrations. Therapeutic targeting of causative oncogenic proteins has remained widely unsuccessful. Essentially, the fetal origin of AML is one of the most distinct unifying features of this heterogeneous group of disorder. We created a hematopoietic atlas to identify fetal stage specific genes and transcripts that are expressed in pediatric AML and in fetal HSCs but are absent from healthy differentiated progenies and adult HSCs. Here, we utilized the CRISPR/Cas9 system to perform pooled library screens on AML cell lines of different subtypes murine fetal HSC based preleukemic model and patient derived xenografts (PDX). We were able to identify novel genes such as the proto-oncogene MYBL2. We further aim to validate these gene candidates and explore their role in leukemogenesis. This work will lay the foundation for developing novel targeted cancer specific therapies, based on this yet unrecognized therapeutic window.

14.00 – 15.30 **X-omics** Chair: Florian Grebien

Acute Myeloid Leukemia initiating cells: identification of new therapeutic targets to avoid relapse

Pau Ximeno-Parpal[1], Vinyoles M[1], Fernández-Fuentes N[1], Martínez-Moreno A[1], Łuczak W[2], Schmid JP[2], Jeremias I[2], Menéndez P[1], Velasco-Hernández T[1]

[1]Josep Carreras Leukaemia Research Institute, Barcelona, Spain; [2]Helmholtz Zentrum München, Munich, Germany.

Relapse in acute myeloid leukemia (AML) is driven by rare therapy-resistant leukemia-initiating stem cells (LSCs) and it is AML's major unsolved clinical challenge. To unravel novel genes involved in LSCs persistence and chemoresistance, we previously generated a comprehensive single-cell expression atlas of AML cells and LSCs in paired diagnostic-relapse, risk-stratified AML patients. We identified the overexpressed genes in LSCs compared to non-LSCs across cytogenetic AML subtypes. To evaluate their effect, we knocked-down (KD) the expression in AML cells and identified a key gene for leukemic progression. We validated these results in vitro, by KD in additional AML cell lines and human primary samples and checked the effect on proliferation and clonogenicity capacity, and in vivo, checking the engraftment capacity and the disease progression of KD cells into immunocompromised mice. Altogether, we have identified a potential target with a crucial role in the AML-LSCs maintenance and survival. Targeting of this gene combined with current standard-of-care therapies might be able to better eliminate both LSCs and bulk population effectively and ultimately avoid relapse in AML.

Targeting the non-coding ands stem cell signature in childhood acute myeloid leukemia

Leah Schüler[1,2,3], Winkler R[1,2,3], Cetin R[5,6], Kaulich M[2], Heckl D[4], Bhayadia R[1,2,3]*, Klusmann JH[1,2,3]*

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Acute myeloid leukemia (AML) is caused by heterogeneous oncogenic events that result in abnormal differentiation of hematopoietic stem (HSC) or progenitor cells. With the aim to identify new leukemia dependencies in the coding and non-coding genome, we bioinformatically compared gene expression signatures from pediatric AML patient samples and healthy donor cells. We could define leukemia subtype-specific gene targets. Further research on those targets using CRISPR-Cas9-based dropout screens will unravel leukemia-specific genes that might play an essential role in genetic aberrations. The results from screens in leukemic cell lines and patient-derived xenografts (PDX) will get a fundamental basis to understand the oncogenic programs driving leukemia. Deciphering the genetic differences between leukemic blasts and healthy HSC helps to invent novel treatments for pediatric AML.

Transcription vulnerabilities profiling through combinatorial CRISPR screens for personalized pediatric cancer therapy

Andrés Carbonell-Adames[1,2], Rassner M[1], Gahleitner L[1], Noll-Puchta H[1], Braun CJ[1,2,3]

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Technical University of Munich, Munich, Germany; 3Hopp Children's Cancer Center Heidelberg (KiTZ), German Cancer Research Center (DKFZ), Heidelberg, Germany

There is an urgent need of precise therapies to treat pediatric brain tumors, as a significant fraction remains fundamentally incurable. Even those successfully cured still suffer from long-term consequences due to the nature of the current treatments. Molecularly targeted therapies that explicitly inhibit tumor-essential processes could led to significant improvement in the survival and quality of life of patients. The emergence of CRISPR technologies delivered a colossal leap forward in novel cancer targets discovery, through massive parallel genetic perturbation screens. Ongoing efforts rely on single gene perturbation screens overlooking the compensatory relationship between genes and pathways. We leveraged a novel combinatorial CRISPR screen approach to extensively evaluate molecular vulnerabilities that arose from dysregulated transcription and associated pathways. Using functional high-throughput CRISPR screens, we identified numerous potential cancer vulnerabilities within transcriptional regulation, which could be used to expedite the design of combinatorial therapies in pediatric glioma and other tumor entities.

Single cell RNAseq uncovering the role of RUNX1/ETO in the leukemic niche signaling

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[1]Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands; [2]Maastricht University, The Netherlands; [3]Wolfson Childhood Cancer Research Centre, Newcastle University, UK.

To study the role of the RUNX1/ETO fusion in interactions of AML cells with the bone marrow niche, we co-cultured mesenchymal stromal cells (MSC) with t(8;21) samples: an AML PDX and bone marrow aspirates from 2 patients. We performed a RUNX1/ETO knockdown (KD) using a lipid nanoparticle siRNA delivery system. Single cell RNAseq (scRNAseq) followed by differential gene expression (DEG) and gene set enrichment analyses suggest that the RUNX1/ETO fusion is necessary for maintaining a pro-inflammatory background in the niche. This is reflected by the downregulation of interferon response and TNF response pathways in MSC upon RUNX1/ETO KD in AML. Preliminary validation of TNF expression in AML upon RUNX1/ETO KD by RT-qPCR supports this hypothesis. Furthermore, scRNAseq revealed an increased expression of extracellular matrix-related genes, such as collagens, in MSC upon RUNX1/ETO KD in AML. This, in turn, may have consequences for the AML progression potential. Together, these data suggest a direct role for leukemic drivers such as RUNX1/ETO in the dysregulation of the leukemic niche and open opportunities for adjunct therapies.

Decoding Epigenetic Landscape in Pediatric Leukemia through Multi-Omics Integration

José Gonçalves-Dias[1-3], Issa H[1-3], Verboon L[1-3], Schuschel K[1-3], Heckl D[4], Bhayadia R[1-3], Klusmann JH[1-3]

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Pediatric leukemia is a complex disease that is driven by epigenetic dysregulation. To gain a comprehensive understanding of the epigenetic mechanisms underlying this disease, we employed a multi-omics approach that integrates data from ATAC-seq, CUT&RUN, and CUT&TAG analyses. By leveraging the strengths of each technology, we obtained a high-resolution view of chromatin accessibility, histone modifications, and transcription factor binding across the genome in pediatric leukemia samples. We aim to depict the complex interactions in the epigenomic mosaic that contribute to pediatric leukemia pathogenesis, identify novel regulatory regions that may contribute to pediatric leukemia pathogenesis, and

identify novel regulatory regions that may play a role in disease development. Overall, our findings can provide new insights into the epigenetic landscape of pediatric leukemia and could inform future efforts to develop targeted therapies.

The transcriptional landscape and the immune microenvironment of the testicular niche in pediatric ALL

Chetan Gunjur Narayanared*dy[1], Pesic M[1], Pfau M[2], Kovacsovics A[1], Yaspo ML[1], Eckert C[2]

[1]Max Planck Institute for Molecular Genetics, Berlin; [2] Charité - Universitätsmedizin Berlin, Germany

Relapse of acute lymphoblastic leukemia (ALL) in children/adolescence occurs in half of male patients with an extramedullary involvement in the testis. The molecular and cellular mechanisms, why leukemic cells preferentially migrate to and survive in the testis have not been systematically assessed. We aimed at characterizing the testicular and the bone marrow niche of ALL cells. We analyzed testicular and bone marrow samples of male pediatric patients using RNASeq and imaging mass cytometry. Our data reveal the physiological immune suppressive microenvironment in the testis compared to the bone marrow. In contrast, we observed a inflammatory environment because of massive leukemic cell proliferation within the tight organ, but an additional immune suppression by the leukemia. There was an increase in apoptosis suppression and DNA repair compared to the bone marrow. We identified different pathways upregulated, supporting the survival of leukemia cells in the testis. In conclusion, the leukemia infiltrated testis is a unique physiologically and pathophysiologically microenvironment, which explains the preferential survival and chemotherapy resistance of in this compartment.

SarcDBase: a tool for detection of genetic alterations in sarcoma

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1 Lund University, Lund, Sweden; 2 Karolinska Institute, Solna, Sweden; 3 Technische Universität München, Munich, Germany; 4 University Hospital and University of Basel, Basel Switzerland.

Sarcomas are a diverse group of malignant tumors arising in the bone and soft tissues and can affect patients of any age. For an increasing number of subtypes, there are already pathognomonic genetic alterations described. However, such alterations remain to be identified for other sarcomas, primarily those with a massive amount of genomic copy number and structural alterations. We have developed SarcDBase, a database-building method that matches genetic variants detected in high-resolution genomic and transcriptomic data from tumour biopsies with information on established biomarkers, eliminating the need for prior knowledge and manual screening of data. The performance of the database was tested on genomic and transcriptomic data from a diverse cohort of sarcomas (n=50). SarcDBase was able to detect mutations that confirmed the diagnosis of some patients, such as NAB2::STAT6, typical of solitary fibrous tumor, and H3F3A p.G35W, typical of giant cell tumor of bone. It was also able to identify new genetic alterations of likely biological significance, such as new combinations of partner genes in gene fusions. SarcDBase can be used on any samples with deep sequencing data.

16.00 – 18.00 **Biomarkers and diagnostics** Chair: Julia Skokowa

PROM1/CD133 expression identifies highly proliferative MLL-AF4+ blasts and correlates with a stem-like gene signature

Joe Cross, Smith AL, Jackson TR, Harman JR, Ling, RE, Crump N, Elliott NE, Wu QQ, Milne TA, Roy A

MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, Department of Paediatrics, University of Oxford, UK.

MLL (KMT2A) rearrangement occurs in 70-80% of infant acute lymphoblastic leukaemia (MLLr iALL), most commonly with AF4 (AFF1). The MLL-AF4 protein activates key leukaemogenic genes, including a stem cell gene PROM1, which encodes the membrane glycoprotein CD133. PROM1 expression in MLLr ALL is higher than in non-MLLr ALL, however there is significant heterogeneity of expression in MLLr iALL patients. Patients with high PROM1 expression have a worse prognosis. Previous work in SEM cell line identified PROM1 as a direct target of MLL-AF4 and showed that it was necessary for in vitro growth. Here we show that PROM1 is bound by MLL-AF4 in primary MLLr ALL samples and in a human fetal liver derived model of MLL-AF4 iALL (CRISPR-MLL-AF4+ ALL). CRISPR-MLL -AF4+ ALL show PROM1 heterogeneity, as seen in patients. CD133+/hi blasts are more proliferative in vitro and in vivo. RNAseq data show CD133+ CRISPR-MLL -AF4+ proB ALL to be enriched for stem-cell and myeloid gene signatures at the expense of lymphoid programmes. Our data suggest that CD133 positivity identifies a high-risk subset of MLLr iALL, and understanding the downstream effects may help identify more appropriate treatments.

IKZF1plusplus? Investigating the genetic complexity of IKZF1 deleted B-cell acute lymphoblastic leukemia

Jonathan Lukas Lühmann[1], Hofmann W[1], Bergmann AK[1], Möricke A[2], Cario G[2], Schrappe M[2], Schlegelberger B[1], Zimmermann M[3], Stanulla M[3], Steinemann D[1]

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Acute lymphoblastic leukemia (ALL) is the most frequent pediatric cancer. Recently, the IKZF1plus profile, defined as the presence of a deletion in IKZF1 together with one or more additional deletions, was characterized as a very poor prognostic marker. This retrospective study aimed to decipher the underlying molecular complexity of the IKZF1plus profile by reanalyzing 142 patients with IKZF1plus or IKZF1 deletion with optical genome mapping (OGM). In 45.9% of the patients either known prognostic markers (18/135 with ETV6::RUNX1, high hyperdiploidy or iAMP21) or other gene fusions (44/135) were identified. The fusions were evenly distributed among the subgroups and categorized into: ABL-class (12), PAX5 (9), JAK2 (10), ZNF384 (4) and other fusions (9). Investigating the outcome, an inferior 5-year event-free survival was observed in presence of a gene fusion (55.9±7.6), especially ABL-class (41.7±14.2), JAK2 (60.0±15.5) and PAX5 (50.0±17.7) fusions when compared to no fusions (70.5±5.4). By using OGM, we show that ~46% of the IKZF1del/plus patients carry a recurrent marker or other gene fusions which may be the underlying leukemogenic driver and contributing to the outcome.

Design of metal-binding proteins for radiotracing and immunoradiotherapy applications

Kateryna Maksymenko, Andreas Muarer, Julia Skokowa, Mohammad ElGamacy

Department of Oncology, Hematology, Immunology, Rheumatology and Clinical Immunology, University Hospital Tübingen, Tübingen, Germany

Homing of radionuclides towards a target antigen, cell, or tissue is an important goal for radiodiagnosis and radioimmunotherapy of a range of cancer types. Radionuclide-conjugated antibodies are thus used to either image or destroy the targetted cancer tissue. Specifically, radioactive Cu(II) isotopes are typically conjugated onto a protein by means of the chemcial coupling of a chelating agent. This chemical coupling process constitutes additional processing steps and entrains positional (i.e. labeling site) and stoichiometric (i.e. labeling ratio) heterogeneity of the resulting conjugate, which lowers their fidelity for clinical applications. Replacing the chelating agent with a metal-binding protein can overcome these challenges, and function as a genetically-encodable radiolabeling tag. However, such proteins must possess sufficient thermal and proteolytic stability, and slow rates of metal dissociation. Hence, we sought different design strategies to create high-affinity, copper-binding proteins. Through design, we also explore the structural determinants of binding and stability of these proteins, which can greatly simplify radiolabeling procedures, and even allow the direct labeling of genetically-modified cell therapies.

Comprehensive molecular and clinical characterization of DUX4-rearranged B-acute lymphoblastic leukemia

Charlotte Friederike Schröder[1], Antić Ž[1], zur Stadt U[2], Tang M[1], Zimmermann M[3], Eckert C[4], Fedders B[5], Stanulla M[3], Cario G[5], Bergmann AK[1]

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B-cell acute lymphoblastic leukemia (B-ALL) with rearrangements involving DUX4 genes (DUX4r) occurs in 4-7% of B-ALL. Due to high variability of the breakpoint loci, DUX4r is difficult to detect during routine diagnostic. This study aimed to characterize DUX4r ALL. Gene expression profiling resulted in a cohort of 132 DUX4-positive cases. Patients were stratified in standard (6%), medium (48%) and high risk (60%) treatment groups according to the ALL-BFM study protocols. In total, 89% of patients achieved remission, 4% relapsed and 5% were lost during follow-up. After induction, 91% were MRD positive (MRD1) and 8% negative, while 47% of patients were MRD-positive after consolidation (MRD2) and 46% negative. Initially relapses were stratified as medium (40%) and high (60%) risk with positive MRD1 (100%) and MRD2 (80%). Using targeted DNA sequencing of available samples, we confirmed DUX4r and identified fusion partners in 72% of cases, with breakpoints in DUX4L2 (28%), DUX4L3 (14%), IGHJ6 (15%) and IGHD7 (12%) genes being the most common. Despite high numbers of MRD2 positive cases, DUX4r relapses are rare and patients might benefit from treatment de-escalation.

Identification of novel fusion transcripts in Acute Myeloid Leukemia

Schuschel K[1-3], Issa H[1-3], Verboon L[1-3], José Gonçalves-Dias[1-3], Vermunt M[1-3], Heckl D[4], Bhayadia R[1-3], Klusmann JH[1-3]

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Acute myeloid leukemia (AML) is a heterogeneous clonal disorder, caused by genetic aberrations that enhance self-renewal and proliferation, block differentiation, and inhibit apoptosis.

Fusion genes (FGs) are main drivers of leukemogenesis, but their variety and occurrence in rare pediatric AML subgroups is yet incompletely defined. Whole transcriptome sequencing, followed by bioinformatical processing provides a powerful tool for analyzing FGs. Here we are utilizing a fusion detection pipeline to study and evaluate 300 human blood samples of pediatric AML patients and healthy donors. We are able to confirm the cytogenetic assessment of leukemic samples and classify not further specified samples and define new undescribed fusions. One of these fusions has MYB as a fusion partner. Novel fusions will be further assessed by molecular genetic assays to evaluate their influence on leukemogenesis and reveal new underlying mechanisms.

Functional Characterization of potential diagnostic targets in Burkitt-NHL

Marcel te Vrugt, Bartels A, Feldmeyer L, Hollfoth V, Tölle I, Randau G, Michgehl U, Hotfilder M, Lanvers-Kaminsky C, Burkhardt B

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Burkitt lymphoma (BL) is the most common malignancy among pediatric Non-Hodgkin Lymphoma (NHL) patients making up for more than 80% of pediatric B-NHL cases. The primary survival rate of sporadic BL (sBL) today exceeds 90% however in case of relapse or progression during therapy the survival rate drops below 30%. The administered intensive chemotherapy regime results in a high incidence of side effects and potentially in second malignancies. Molecular-based risk stratification of sBL is urgently needed. In a previous study of our group, the genetic material of 191 pediatric patients, of which 30 suffered from relapse, registered within the NHL-BFM study center was analyzed via a targeted resequencing approach. Several recurrently mutated genes including hotspot mutations in TP53, FBXO11/ FOXO1 and PCBP1 were identified. Patients with TP53 wild type status and variants in FBXO11mt and/or FOXO1mt are associated with a lower incidence of relapse (p=0,05). Also TP53mt significantly increased the cumulative incidence of relapse (p=0,0002). Recent studies support the finding that TP53mt correlate with a higher risk for progression, relapse, and a lower event-free survival in sBL.

Identification of cell-extrinsic pro-survival factors in JMML

Anton Wehner, Koleci N, Rajak J, Wang J, Xiao H, Erlacher M

UNIVERSITÄTSKLINIKUM FREIBURG, Zentrum für Kinder- und Jugendmedizin, Klinik für Pädiatrische Hämatologie und Onkologie, Forschungslabor Hämatologie AG Erlacher

Juvenile myelomonocytic leukemia (JMML) is an aggressive early childhood myeloid leukemia caused by constitutive RAS pathway activation, with the most severe form showing a mutation in the PTPN11 gene/SHP2 tyrosine phosphatase. Our previous work has shown that expression of an oncogenic SHP2 protein in the murine hematopoietic system results in apoptosis resistance of the leukemic myeloid compartment and that environmental signals further improve cell survival. Mass spectrometry revealed CD74 and CD11b as candidate molecules possibly contributing to the apoptosis resistance. CD74 is a surface receptor promoting cell proliferation and survival. Integrin CD11b regulates adhesion and migration. We investigated which JMML cells express CD74 and CD11b and if inhibition of either molecule promotes survival in these cells (MxCre;Ptpn11D61Y/+). We found CD74 upregulated in leukemic neutrophils, macrophages and dendritic cells but not in JMML stem and progenitor cells. CD11b was upregulated specifically in monocytes. However, in vitro blocking of either CD74 or CD11b did not result in apoptosis. We therefore hypothesize that both molecules play other, e.g. immunomodulatory functions in JMML

SATURDAY, JUNE 3rd

09.00 – 10.15 **Malignant cell biology** Chair: Eva Rettinger

Extracellular vesicles transfer chromatin-like structures that induce non-mutational dysfunction of p53 in bone marrow

Jamal Ghanam, Chetty VK, Reinhardt D, Thakur BT

Department of Pediatrics III, University Hospital Essen, Essen.

Acute myeloid leukemia (AML) is the second most common leukemia. Despite the significant progress in AML therapy, little is known about how leukemia cells alter the bone marrow niche to facilitate their growth and evade chemotherapy or relapse after successful treatment. The tumor suppressor p53 has been highly expressed in the stem cells compartment, leading to high expression of p21 that induces cell cycle arrest required to maintain the stem cell property. We have identified a chromatin-like structure we termed EV-chromatin as a novel component of small EVs. EV-chromatin represents a mixture of DNA and proteins, such as histones and S100 proteins (S100A4, A8, A9, and A16). Interestingly, EV-chromatin isolated from leukemic blasts has the capacity to alter the proliferation of bone marrow mesenchymal stem cells (BM-MSCs). Mechanically, our data suggest that leukemic EV-chromatin downregulates the p53-mediated transcription of p21. This was accompanied by a significant increase in MDM2 levels, suggesting a p53-mediated decrease of p21, BAX, and PUMA. Conversely, treatment with the MDM-2 inhibitor Siremadlin rescued the p53 transcriptional activity in BM-MSCs.

CSF1R as a marker of lineage plasticity in MLL-AF9 infant leukaemia

Giuseppina Camiolo, Ottersbach K

Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, UK

MLL-AF9 translocations can cause either AML or B-ALL in infants. The haematopoietic progenitor cell in which this choice occurs and the underlying mechanisms are still unknown. Given the reported existence of foetal haematopoietic precursors (LMPPs), characterised by CSF1R expression, that possess dual B-myeloid potential, we sorted CSF1R+ and CSF1R- LMPPs following MLL-AF9 induction to test whether CSF1R influences the lineage choice in the context of MLL-AF9 expression. Our data show that MLL-AF9+CSF1R+ LMPPs are more proliferative and have higher plasticity than CSF1R- cells, and induce a faster disease progression, when injected into NSG recipients. Importantly, only CSF1R+ cells from primary recipients fully engrafted secondary and tertiary recipients, which showed myeloblast infiltration in spleen, liver and peripheral blood. Surprisingly, in one of our recipients we saw for the first-time a mixed phenotype and potential lineage switching, confirming higher plasticity of CSF1R+ cells. RNA-seq analysis of MLL-AF9+ CSF1R+/- LMPPs is ongoing to define transcriptional differences, and to find and validate gene candidates driving lineage plasticity in MLL-AF9+ CSF1R+LMPPs.

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

Vera Muench[1], Koehler R[2], Rasche V[3], Debatin KM[1], Meyer LH[1]

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Understanding the precise dynamics of ALL engraftment in patient-derived xenograft (PDX) mouse models is of major interest to preclinically evaluate novel directed therapeutics. We analyzed ALL engraftment in peripheral blood (PB), bone marrow (BM), spleen, and central

nervous system (CNS) in the NOD/SCID/huALL mouse model over time using FACS to detect human CD19, MRD marker quantification, and cranial magnetic resonance imaging (MRI). Using FACS or MRD, ALL infiltration was first detected in BM and last in PB. Applying MRD, BM-ALL was detected 1-2 weeks earlier than by FACS reflecting the higher sensitivity of this method. Most interestingly, first CNS-ALL with positive MRD was detected early on, in some mice even along with BM-ALL and earlier and/or at higher levels as compared to first spleen-ALL. MRI showed first circumscribed CNS infiltrates 1-2 weeks after FACS detection and clearly enlarged meninges later on corresponding to the macroscopic presentation upon autopsy. In summary, our findings on very early CNS-ALL manifestation indicate a high and early propensity of ALL cells to manifest in the CNS and contribute to the understanding of ALL biology.

AML-derived small EVs containing YBX1 influences mesenchymal stromal cells differentiation in the bone marrow niche

Venkatesh Kumar Chetty, Ghanam J, Reinhardt D, Thakur BK

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Only recently, it is known that small extracellular vesicles (sEVs) released by AML cells induce molecular changes in the bone marrow niche (BMN) and transform BMN into leukemia permissive niche. However, it remains unclear which biological cargo from AML-derived sEVs has a functional role in BMN. We found that MV4-11 sEVs influence the normal hematopoietic function in healthy bone marrow-derived mesenchymal stromal cells (BM-MSCs) leading to increased proliferation and decreased differentiation. Next, LC-MS proteomics revealed that many proteins including YBX1 are upregulated in both untreated MV4-11 sEVs and healthy BM-MSCs treated with MV4-11 sEVs. Supporting this, we found that YBX1 is significantly upregulated in AML patients-derived sEVs compared to healthy controls. Interestingly, incubation of healthy BM-MSCs with sEVs isolated from MV4-11 cells with pharmacological YBX1 inhibitor or downregulating YBX1 using siRNA conditions significantly rescued the observed effect of proliferation and differentiation. Altogether, we revealed that YBX1 is a novel protein in AML-derived sEVs, which disrupts normal hematopoiesis in BMN by influencing the differentiation of BM-MSCs.

NG2 and VLA-4 are involved in the invasion and migration of MLLr B-cell Acute Lymphoblastic Leukemia

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B-cell Acute Lymphoblastic Leukemia with MLL gene rearrangements (MLLr B-ALL) is an aggressive subtype of B-ALL (overall survival< 40%), whose patients are usually younger than 1 year and present therapy resistance and high relapse rates. Remarkably, the proteoglycan Neuron-glial antigen 2 (NG2), which is barely expressed in normal hematopoietic cells, is

expressed in the leukemic cells of around 90% of MLLr B-ALL patients. However, its role in MLLr B-ALL remains elusive. In recent years, our group has correlated NG2 expression with poor prognosis and central nervous system (CNS) infiltration. Importantly, NG2 has been associated to migration through its interaction with integrins in solid tumors. Our data showed a co-localization of NG2 and integrin α4β1 (ITGA4/ITGB1) by imaging flow cytometry, as well as a higher expression of ITGA4 in NG2-positive sorted blasts, suggesting a cooperation between both proteins. Finally, our in vivo results revealed that mice transplanted intravenously with NG2/ITGA4 double knock-out MLLr B-ALL cell line present long delay in the development of leukemia, suggesting that ITGA4 and NG2 could be cooperating in homing and migration of leukemic cells.

10.45 – 11.45 **Molecular mechanisms of disease II** Chair: Basant Thakur

Optimizing CRISPR/Cas9 technologies to develop disease model systems

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The CRISPR/Cas9 system is widely used as state of the art genetic engineering to create specific disease models *in vitro*. We used this system to mimic leukemic chromosomal translocations like KMT2A-AFF1 t(4;11) and KMT2A-MLLT4 t(6;11) in K562 cells and HSPCs. It turned out that many aspects have to be considered regarding the transfection parameters and culture conditions. Interestingly, the K562 cell line was not only suitable to check sgRNA efficiency, we were also able to induce both chromosomal translocations, the t(4;11) and t(6;11), in K562 cells with CRISPR/Cas9. The K562 cells are undifferentiated blast cells growing in suspension and dividing rapidly caused by a *bcr-abl* fusion. Due to the high cell proliferation, the NHEJ repair mechanism appeared as preferential repair method after double strand break induction. Is the proliferation status of HSPCs also mandatory to induce chromosomal translocations or is it just donor dependent? Future perspectives will be discussed.

Identification of Functional Defects Leading to Bone Marrow Failure in GATA2 Deficiency

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Hematopoiesis is regulated by several transcription factors that ensure both a proper blood cell production and the survival of immature hematopoietic stem and progenitor cells (HSPCs). Among them, GATA2 plays an essential role in HSPC development and differentiation, controlling gene transcription of multiple target genes. Disruption of this balance caused by germline monoallelic GATA2 mutations leads to variable phenotypes like immunodeficiency, cytopenia, lymphedema and others. Patients are at high risk to develop myelodysplastic syndrome or acute myeloid leukemia. We previously established a transgenic mouse model for GATA2 heterozygosity and showed that the transplantation of Gata2+/- HSPCs into lethally irradiated mice induced bone marrow failure and secondary leukemia. Gata2+/- HSPCs show poor engraftment within the first weeks after transplantation due to an increased apoptotic susceptibility. In vitro experiments showed that Gata2+/- HSPCs are more sensitive towards serum deprivation and the pan-kinase inhibitor staurosporine. We are currently characterizing apoptosis signaling in Gata2+/- HSPCs in more detail and focus on various stress signals including kinase inhibitors

TP53 separation-of-function mutations through promoter swapping in osteosarcoma

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TP53 is a tumour suppressor gene but typically shows recurrent missense mutations or retained structural alterations in cancer, indicative of a concurrent loss- and gain-of-function mechanism. Structural variants in TP53 often result in loss of the coding parts of the gene while simultaneously preserving the promoter region. This phenomenon is particularly common in osteosarcoma, the most common primary bone malignancy. To unravel the consequences of a TP53 promoter relocated in this manner, we performed in-depth genetic analyses of osteosarcoma biopsies and cell models. We show that TP53 structural variants are early events that denote a subgroup of young osteosarcoma patients, frequently associated with positive selection for the TP53 promoter and a high breakpoint burden. Furthermore, the active TP53 promoter region paradoxically upregulates genes significantly associated with the TP53 signalling pathway itself. This suggests that while tumor supressor actitivites of the TP53 pathway are lost, survival and proliferative features are retained. Our findings demonstrate a need to counterbalance loss of TP53 function through separation-of-function mutations via promoter swapping.

Genotype-phenotype correlations in patients with severe congenital neutropenia and cyclic neutropenia harboring *ELANE* mutations

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Background:

Severe congenital neutropenia (CN) and cyclic neutropenia (CyN) are bone marrow failure syndromes with a high risk of progression to acute myeloid leukemia (AML). The most frequent pathogenic defects found in CN/CyN are heterozygous mutations in the *ELANE* gene encoding for neutrophil elastase.

Over 120 distinct *ELANE* mutations have been described to date. Recently it was shown that the diagnosis of CyN is associated with mutations in exon 4, intron 4, and exon 5. Several variants (i.e., p. G214R, p. C151Y) were associated with a higher risk of AML. However, specific *ELANE* mutations have limited predictive value for leukemogenesis, and there is a lack of data on the mutations leading to poor response to G-CSF.

Moreover, while all CN/CyN patients previously described in the literature harbored heterozygous *ELANE* variants, there are no data on how homozygous mutations in this gene may affect the clinical phenotype.

Aims:

The study aims to assess the relationships of different *ELANE* mutations and clinical outcomes in patients with CN and CyN.

Methods:

We evaluated data on patients with CN/CyN referred to the Severe Congenital Neutropenia International Register, Dmitry Rogachev National Medical Research Center, and Alexandria University Children's Hospital. The data included clinical records, family history, the results of genetic tests, blood and bone marrow investigations. The clinical status and treatment responses were reviewed annually using standard forms. Study approval was obtained from the Ethical Review Board of the Medical Faculty, University of Tübingen.

Results:

In total, 239 patients with 99 distinct *ELANE* mutations were included in this study. The mutational pattern differed between CN and CyN. Several mutations were strongly associated with CyN (i.e., p.P139L, p.P220Q) or CN (i.e., p.I120F, p.C151S, p.G214R) only. However, most of the mutations overlapped between these two groups. We identified *ELANE* mutations associated with poor response to G-CSF therapy (median dose ≥20 µg/kg per day): p.M1K, p.P42L, p.A57V, p.C151S, p.W156C, and p.G214R. Several mutations were strongly associated with a high risk for AML development: p.S126L, p.C151Y, p.G214R, and p.D230Mfs*10. The percentages of poor responders to G-CSF and cases of AML were higher in CN compared to CyN. However, in our cohort, there were 2 AML cases in patients with CyN harboring different *ELANE* mutations.

We also identified an Arab family whose members carried both pathogenic *ELANE* p.R220Q mutation (previously described in patients with CyN), and p.P257L benign polymorphism. To date, we have examined 54 out of 116 members of this family. Among them, 21 subjects were heterozygous, and 4 subjects were homozygous for both *ELANE* variants. All homozygous subjects had severe neutropenia and presented with recurrent attacks of fever, mouth ulcers, and severe infectious complications. Interestingly, clinical phenotypes were highly variable among the heterozygous subjects, and several of them might be considered asymptomatic carriers.

Summary/Conclusion:

This study identified clinically important genotype-phenotype correlations in patients with CN or CyN harboring *ELANE* mutations. We found several mutations associated with a relatively good and with poor prognosis for response to G-CSF and the development of AML. We also reported a family with family members carrying homozygous or heterozygous *ELANE* mutations for the first time. This analysis provides valuable information to guide clinical care for patients with CN and CyN.