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0001 RACK1 as a novel therapeutic target against Acute Myeloid Leukemia stem cells

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Chemoresistant and self-renewing leukemia stem cells (LSCs) have been found to be the underlying cause behind acute myeloid leukemia (AML) relapse after standard-of-care treatment. Achieving successful elimination of LSCs in AML patients is an unmet challenge, that could lead to a more long-lasting clinical response, increasing survival and, ultimately, avoiding disease relapse. To describe novel regulatory proteins with therapeutic potential against AML-LSC, our group has recently performed a single-cell-RNA sequencing analysis on samples from AML patients, identifying several differentially overexpressed genes on LSCs. The bioinformatic screening and functional assessment of these genes revealed RACK1 as a promising candidate, with consistent loss-of-function effects when downregulated through shRNA technology in AML cell lines and primary samples. Although it has been associated with several oncogenic pathways in solid tumors, RACK1's role in blood malignancies remains poorly described. To our knowledge, this is the first exhaustive assessment of RACK1 therapeutic potential for targeting AML-LSCs.

0002 Characterization of childhood B-ALL cell lines to identify novel CIN-targeted therapies

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B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood cancer, with 30-40 % of cases exhibiting aneuploidy in leukemic cells. Aneuploidy is linked to chromosomal instability (CIN), characterized by frequent chromosome segregation errors during cell division. Here, we aim to identify candidate genes associated with CIN tolerance and characterize B-ALL cell lines as in vitro models for functional validation.
We conducted RNA-Seq and mass spectrometry on patient-derived xenograft (PDX) B-ALL samples, identifying 81 genes positively correlated with CIN. Next, we characterized CIN and mitotic defects in aneuploid B-ALL cell lines MHH-CALL2 and NALM16, using the t(4;11) SEM cell line as a control. Confocal live-cell imaging revealed longer prometaphases and increased chromosome segregation errors in NALM16, confirmed using the Opera Phenix microscope and a semi-automated ImageJ plugin. CIN levels correlated with chromosome copy number heterogeneity, with NALM16 showing higher deviations. Furthermore, TP53 and P21 activity were absent in NALM16 and SEM but detectable in MHH-CALL2. Our study characterizes B-ALL cell lines, highlighting distinct CIN rates and TP53 activity

0003 Towards new therapeutic strategies in t(4;11) leukemia

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The current treatment of t(4;11) ALL is still correlated with poor outcome. Infant t(4;11) ALL is even worse. Thus, there is a medical need to improve the standard CTX or BMT. This can only been done with the molecular understanding of this disease entity. Here, we are following a therapeutic approach that has been brought up in two different laboratories (Barcelona and Frankfurt). The molecular targets are the KMT2A-AFF1 fusion protein and the Polycomb repressor complex II (PRC II) constituent EZH1/2. Both can be targeted by small molecules Entinostat and UNC1999, respectively. Entinostat is a selective HDAC class I inhibitor (HDAC 1-3) that disables KMT2A-AFF1 to bind to its target genes but enhances binding of wildtype KMT2A. UNC1999 inhibits the histone modifying enzymatic domain of EZH1/2, the key components of PRC II. The combination of both drugs enable chromatin modifications without targeting the biological activity of either KMT2A or AFF1 that are essential for living cells. We will present our data about single-agent or combined treatments in a t(4;11) model system and will discuss the potential consequences for future therapeutic interventions.

0004 The induction of CRISPR/Cas9-mediated KMT2A-rearrangements in umbilical cord blood HSPCs in vitro

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KMT2A-rearranged (r) chromosomal translocations cause high risk pediatric, childhood and adult acute lymphoblastic and myeloid leukemia. By applying our recently established CRISPR/Cas9 system, we were able to induce t(4;11), t(6;11), t(9;11) and t(11;11) chromosomal translocations in healthy umbilical cord blood hematopoietic stem and progenitor cells (HSPCs) and established a long term ex vivo cell culture system that allowed the outgrowth of KMT2A-r cells and monitoring the molecular transition into pre-leukemic cells. In case of t(4;11) HSPCs, the addition of lineage specific cytokines shifted these KM-

T2A-r cells either into a pro B or myeloid direction, demonstrating their plasticity for lineage-specific outgrowth. Our ex vivo model system allowed to recapitulate the transition from healthy to pre-leukemic cells over a short period of time. MACE-Seq data confirmed this transition and showed the up-regulation of lineage-specific target genes during 70 days of ex vivo culture.

0005 Mechanisms of cell death induced by NK cells in DLBCL

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The clinical application of CAR-T or NK cells demonstrates the potential of cellular immunotherapy as treatment option in different malignancies, including Diffuse Large B-cell Lymphoma (DLBCL). NK cells kill tumor cells by different mechanisms to trigger apoptotic and necrotic cell death within the targeted cells. We and others have recently shown that also the intrinsic apoptotic pathway is activated by NK cells, and that BH3-mimetics may sensitize for NK cell-mediated killing in solid tumors. However, the precise mechanisms of NK cell-mediated killing and the importance of the different cell death pathways are currently not understood. In this study, we characterize NK-cell induced cell death pathways in DLBCL. Our data highlight the importance of TRAIL-signalling and death receptor-induced apoptosis. In contrast, while mitochondria were clearly perturbed during NK cell attack, the intrinsic apoptotic pathway does not appear to be required for killing by NK cells. In addition, BH3-mimetics able to alleviate mitochondrial apoptosis inhibition did not synergize with NK cell killing. Current studies are investigating combination treatments able to optimize NK cell killing.

0006 Investigating the Potential of BH3 Mimetics and NK Cell Immunotherapy in Pediatric Sarcoma Treatment

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Rhabdomyosarcoma (RMS) is the primary soft tissue cancer in children, with poor survival rates despite improvements. To uncover improved treatment alternatives, in vitro models that accurately reflect the tumors are necessary. Therefore, we established a pipeline to develop primary patient-derived cells as new models for drug and immunotherapy testing. Preliminary results obtained in primary 2D monolayer and 3D spheroids show that RMS cells are sensitive to BCL XL and MCL-1 inhibitors, as well as to attack by activated allogenic NK cells. Killing by NK cells was further increased by expression of a B7H3-CAR construct. Additionally, these cells were validated for use in a patient-derived xenograft (PDX) model in vivo. To further build on these initial discoveries, we are currently performing larger drug screening approaches as well as mechanistic studies. Taken together, we aim to validate the use of primary cell-derived tumor spheroids as models of sarcoma and establish a platform for the development of novel therapeutics with combination of BH3 mimetis with NK cell-based immunotherapy.

0007 Targeting senescence as anti-lymphoma strategy

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Cellular senescence is a naturally occurring growth arrest within aging tissues that can be triggered by factors like oncogenes or chemotherapy. Some anti-cancer drugs induce both apoptosis and therapy-induced senescence (TIS), which is associated with treatment resistance and relapse in solid tumors. While TIS initially prevents tumor cell proliferation, it can contribute to tumor progression by influencing surrounding tumor cells through the secretion of factors referred as the senescence-associated secretory phenotype. The role of TIS in hematologic cancers, like diffuse large B-cell lymphoma (DLBCL) remains poorly characterized. Around 40 % of DLBCL patients relapse or suffer a refractory disease, highlighting the importance of understanding resistance mechanisms. In this study, we investigate which drugs may trigger senescence in DLBCL, what effects senescent cells have on surrounding cells and how they react to apoptosis inducers. First experiments show that doxorubicin, part of the treatment of DLBCL, induces both senescence and apoptosis in DLBCL cells. Ongoing experiments aim to elucidate whether the accumulation of senescent cells contributes to resistance in DLBCL.

0008 Rapid Epigenomic Classification of Acute Leukemia

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Acute leukemias (AL) require precise molecular classification and urgent treatment. However, standard-of-care diagnostic tests are time and resource intensive and do not capture the full spectrum of AL heterogeneity. Here, we developed a framework to classify AL using genome-wide DNA methylation profiling. We first assembled a large reference cohort (n = 2,540 samples) and defined 38 methylation classes across lineages and age groups. Methylation-based classification matched lineage classification by standard pathology evaluation in most cases and revealed heterogeneity beyond that captured by genetic categories. Using this reference, we developed a neural network (MARLIN) for AL classification from sparse DNA methylation profiles. In retrospective cohorts profiled by nanopore sequencing, high confidence predictions were concordant with conventional diagnoses in 25/26 cases. Real-time MARLIN classification in patients with suspected AL resulted in accurate predictions in 5/5 cases, which were typically generated in less than two hours from sample receipt. In summary, we present a novel framework for rapid AL classification that can complement and enhance standard-of-care diagnostics.

0009 A quiescence/TGF-β1 CRISPRi screen reveals drug uptake transporters as secondary targets of kinase inhibitors in AML

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Acute myeloid leukemia (AML) relapse is driven by leukemia cells that survive treatment. Since standard chemotherapy targets cycling cells, various forms of dormancy, including quiescence, may allow them to evade therapy and trigger relapse. However, the role of quiescence in AML therapy remains unclear. Here, we show that quiescence in AML is strongly associated with poor patient outcomes. It coincides with active TGF-β signaling, and treatment of AML cells with TGF-β1 induces a G0 arrest. A drug-focused CRISPRi screen identified TGFBR1 inhibitors as effective in preventing quiescence. However, although treatment with the TGF-β inhibitor vactosertib prevents quiescence, it comple-

tely abolishes cytarabine-induced cell death. Further investigation, combining a second CRISPRi screen with LC-MS/MS and in silico analyses, uncovered that vactosertib targets the nucleoside transporter SLC29A1 (ENT1), reducing intracellular cytarabine levels. Importantly, we found that this drug interaction is not unique to TGFBR1 inhibitors, but extends to other clinically important kinase inhibitors. These findings may have important implications for optimizing combination therapies in the future.

0010 Analyzing Transcriptomic Alternative Landscapes in Pediatric AML: Insights from Long-Read RNA Sequencing

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Pediatric leukemia, particularly Acute Myeloid Leukemia (AML), exhibits significant molecular heterogeneity driven by the diversity of gene isoforms, alternative splicing events and the presence of fusion genes. Previous studies have highlighted the role of isoform disequilibrium in promoting oncogenesis, such as the RUNX1A/C imbalance, demonstrating the importance of isoform-specific expression patterns in disease pathogenesis. By employing long-read Nanopore RNA sequencing, we aim to accurately identify and quantify full-length transcripts and detect alternative splicing events that are often missed by traditional short-read sequencing methods. This approach allows us to better understand the intricate landscape of pediatric leukemia pathogenesis, its transcript diversity and regulatory mechanisms. Furthermore, we highlight the unique isoform profiles and splicing events in Down syndrome patients with myeloid (ML-DS) or transient abnormal myelopoiesis (TAM) potential role of chromosomal abnormalities in shaping the transcriptome. This comprehensive profiling will provide insights into the molecular profiles of different leukemia subtypes and may uncover novel therapeutic targets

0011 Functional identification and targeting of zinc-finger containing proteins at domain resolution in leukemia

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Genes encoding zinc finger (ZnF) proteins are often aberrantly expressed and/or mutated in acute myeloid leukemia (AML). Dysregulation of these factors can lead to disruption of normal hematopoiesis and promotes leukemogenesis. The prototypic ZnF is a small domain consisting of cysteine and histidine residues that coordinate one or more zinc ions. This structure enables their function to promote direct interaction with DNA, RNA and proteins and the regulation of various cellular processes. Importantly, ZnF domains are emerging drug targets, like in targeted protein degradation by thalidomide analogs. We hypothesize that identifying ZnF domains critical for AML cell growth will provide new entry points for the design of novel therapeutic strategies. In this project, we conduct a base-editor screen targeting all cysteine and histidine residues within all ZnF domains of all human ZnF proteins. We will validate the role of candidate ZnF domains, interaction partners and underlying mechanisms in AML. This will provide insights into the domain-specific functions of ZnF proteins and enable the development of chemical strategies to target specific domains ZnF proteins in AML.

0012 A FRET-based approach to identify critical factors for biomolecular condensation in NUP98::KDM5A-driven AML

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Pediatric AML is a highly aggressive disease with poor outcomes and limited treatment options. In NUP98-fusion AML, the N-terminal region of NUP98 fuses to over 30 partner genes, creating fusion oncoproteins that disrupt myeloid differentiation and lead to aberrant gene expression. We and others have shown that NUP98-fusion oncoproteins form biomolecular condensates in the nucleus, but the factors critical for NUP98::KDM5A condensation and potential targeting strategies of "onco-condensates" remain unclear. To identify and perturb components critical for NUP98::KDM5A condensation we mapped the interactome of NUP98::KDM5A using bioID, identifying 36 druggable proteins specifically interacting with the fusion oncoprotein. To test proximity and interaction strength of NUP98::KDM5A within onco-condensates we established a flow cytometry-based Förster-Resonance-Energy-Transfer (Flow-FRET) assay via co-expression of NUP98::KDM5A tagged with mCherry and eYFP, respectively. This assay enables genetic and pharmacological screens to investigate the effect of candidate proteins on condensate formation, pinpointing potential targets for AML therapy via disruption of onco-condensates.

0013 Identification of germline PAX5 variants in pediatric B-ALL: Establishing murine and hiPSC model systems for profiling

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PAX5 is crucial for B cell development, regulating key differentiation factors like CD19 and CD79a. While PAX5 somatic mutations occur in one-third of B-ALL cases, this study highlights the impact of germline variants. Sequencing data from 1,161 childhood cancer patients revealed four known (R38H, P80R, G183R, G183S) and ten novel (e.g., R31W, G183Afs, E259K, G343R, Exon 6 deletion, stop loss) PAX5 variants. Six (R31W, G183Afs, E259K, G343R, Exon 6 deletion, stop loss) demonstrated functional relevance in vitro, particularly affecting CD19 and CD79a. RNA sequencing showed significant differences in gene expression impacting B cell development. While murine models have limitations, an iPSC-based human system using CRISPR-Cas9, which is currently under development, could provide deeper insights into B-ALL initiation. Taken together, our data suggest a higher prevalence of PAX5 germline variants than previously assumed.

0014 Therapeutic targeting of the epigenetic network of NPM1c in acute myeloid leukemia

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Mutations in (NPM1) are the most prevalent genetic alteration in acute myeloid leukemia (AML), found in nearly one-third of cases, and result in its abnormal localization to the cytoplasm (NPM1c). While its cytoplasmic effects have

been extensively studied, emerging evidence suggests that NPM1c also plays a critical role within the nucleus, where it associates chromatin at key gene loci, such as HOXA/B and MEIS1, contributing to leukemogenesis. We have recently shown that NPM1c cooperates with KMT2A and that this process can be targeted using Menin-MLL inhibition. However, resistance to Menin inhibitors has been described and additional treatment approaches are needed. To explore potential therapeutic strategies, we generated a reporter cell line to track HOXA9 and MEIS1 expressions and conducted a large-scale screen of epigenetic compounds to identify regulators of oncogenic transcriptional programs. Additionally, we assessed the potential for synergistic effects with Menin inhibitors which are already in clinical trial. This approach not only facilitates the discovery of novel epigenetic treatments but also provides insight into new molecular vulnerabilities in NPM1c-driven AML.

0015 Investigating the cooperating factors of NPM1c on chromatin in AML

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Nucleophosmin 1 (NPM1) mutations are the most common genetic lesion in acute myeloid leukemia (AML), found in one-third of cases and leading to the cytoplasmic mislocalization of the protein (NPM1c). Until now, the focus on NPM1c in leukemia was predominantly centred on its cytoplasmic activities. However, we recently showed a pivotal role for NPM1c within the very core of leukemia cells - the nucleus - where it binds to chromatin at important self-renewal-associated gene loci, such as HOXA/B and MEIS1, and directly regulates the oncogenic transcription. Now the critical question is which other nuclear factors cooperate with NPM1c to drive leukemic transformation. Using proximity labelling and mass spectrometry, we identified nuclear pore complex (NPC) proteins as potential cooperating factors of NPM1c. To investigate their functional relevance, we are employing chromatin binding studies, CRISPR-Cas9 editing and targeted protein degradation techniques to determine how nucleoporins cooperate with NPM1c in leukemia development. This study aims to expand our understanding of disease development and thereby identify molecular targets for potential new epigenetic therapies.

0016 Understanding the influence of JAK3 germline vs. somatic variants

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Recent studies suggest that germline predispositions contribute to childhood cancer in up to 15 % of cases. Identifying at-risk children enables preventive strategies. However, most germline variants are classified as variants of unknown significance (VUS). Additionally, it is unclear why genes like Janus Kinase 3 (JAK3) exhibit both germline and somatic cancer-related variations with differing effects. To test this, we cloned 10 novel germline VUS and 6 somatic JAK3 variants identified in hematological malignancies into an inducible JAK3 expression system, allowing their functional validation in Ba/F3 cells through proliferation, signaling, and phospho-flow assays. An IPTG-inducible Jak3 knockdown reduced interference from endogenous Jak3. The germline VUS JAK3 p.Y399C, identified in a cancer-prone family, showed weak constitutive proliferative effects but insufficient oncogenic potential for IL-3 independence. It did, however, upregulate pStat3, unlike the somatic Jak3 p.V670A variant, which downregulated pStat3 and pAkt. These findings highlight differences in germline vs. somatic JAK3 variants, aiding understanding of JAK3-driven childhood hematological malignancies.

0017 Digenic Heterozygous Variants in FANCF and FANCM Disrupt the Fanconi Anemia Pathway in Childhood Leukemia

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Germline variants in DNA repair genes play a crucial role in pediatric cancer predisposition. In our TRIO study (n = 181), which analyzes exomes of children with cancer and their parents, we identified rare heterozygous digenic variants in Fanconi anemia (FA) pathway genes. As most FA genes are autosomal recessive, single heterozygous variants are not expected to be pathogenic. Here, we specifically investigated one B-ALL patient carrying truncating variants in FANCF and FANCM, each inherited from a parent. To assess their functional relevance, we generated HEK293 cell models using CRISPR-Cas9 mediated knockout of endogenous FANCF/FANCM expression and retroviral overexpression of wild-type and mutant alleles. DNA damage response was analyzed via γH2AX foci after irradiation, and protein interactions were examined through co-immunoprecipitation. Cells with both variants showed impaired DNA repair and altered FA-core complex interactions. These findings support a model in which heterozygous digenic FANCF/FANCM variants disrupt FA signaling and contribute to leukemogenesis. Our data emphasize the need to consider digenic inheritance in cancer genetics and diagnostics.

0018 Relapse Mechanisms in Children with Down Syndrome and Myeloid Leukemia

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Children with Down syndrome (DS) achieve high cure rates with frontline therapy but are hypersensitive to cytarabine. Yet, relapse remains a major clinical challenge upon which event-free survival rates drop below 20 %. To overcome toxicity while maintaining efficacy, ML-DS 2018 clinical trial replaced idarubicin, cytarabine, and etoposide (ML-DS 2006) with CPX-351, a liposomal formulation of cytarabine and daunorubicin in a 5:1 molar ratio. To evaluate treatment response and relapse mechanisms, patient-derived xenografts (PDXs) were established from initial diagnosis and relapse samples across both trials. These models underwent comprehensive in vitro testing, confirming the CPX-351 efficacy. Orthogonal multi-omics integrative strategies (OMCIS) were applied to identify relapse-associated molecular alterations, while single-cell RNA sequencing (scRNA-seq) was used to detect chemoresistant subclones. By systematically comparing PDX samples from initial diagnosis and relapse, this study will identify key drivers of therapy resistance to uncover novel treatment vulnerabilities and support the development of more effective therapeutic strategies for relapsed ML-DS patients.

0019 CRISPR-based optimization of CAR NK cell therapy for pediatric AML

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Natural Killer (NK) cells are great candidates for an allogeneic “off-the-shelf” therapy in pediatric acute myeloid leukemia, due to their innate effectiveness against cancer cells without causing graft-versus-host disease or cytokine-release syndrome. This project aims to enhance anti-tumor activity of NK cells through CRISPR-Cas9 mediated gene editing. However, efficient transduction of NK cells with lentiviral vectors using vesicular stomatitis virus glycoprotein

(VSV-G) remains challenging. Following different routes to improve NK cell editing we apply pseudotyping with synthetic VSV-G mutants co-expressing NK cell-targeting ligands or scFvs, electroporation to deliver Cas9, lipid-nanoparticle based Cas9-mRNA transfer and peptide-enabled Cas9 protein-transduction to achieve gene editing along with a custom sgRNA library to identify genes influencing NK cell activity, proliferation and persistence. Overall, we aim to establish a more reliable and efficient platform to transduce NK cells and improve NK cell-based cancer immunotherapy.

0020 Therapy-induced senescence shapes tumor biology and treatment outcome in hepatoblastoma

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Hepatoblastoma (HB) patients with high-risk disease show poor survival and require better treatment options. Due to few recurrent genetic aberrations targeted therapies remain unavailable. Characterizing dynamic cell state changes in response to chemotherapy, such as therapy-induced senescence (TIS), may unveil new targets for synthetic lethal combination treatments. Our goal is to identify TIS-specific vulnerabilities for "one-two punch" therapies using chemotherapy and senolytic drugs. HB cell lines (HepG2, Hep293TT, HUH6), PDX models, and Myc-/Ctnnb1-driven tumor mouse models were treated with doxorubicin and cisplatin. TIS was confirmed by H3K9me3/Ki67 immunofluorescence (IF) and SA-β-gal staining. In murine HB RNA sequencing and spatial IF imaging revealed senescence-associated phenotypes, such as an increased immunogenicity alongside an enhanced macrophage and T-cell infiltration. Using a large pharmacological inhibitor screen, we identified the ERAD pathway as a target for senolytic therapies. We found that TIS influences tumor biology and treatment, making senolytic 'one-two punch' therapies a promising strategy for high-risk hepatoblastoma.

0021 Elucidating the role of Exportin-1 (XPO1) in NPM1c-driven acute myeloid leukemogenesis

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The Nucleophosmin 1 (NPM1) gene encodes a versatile nucleolar protein that shuttles between the nucleus and cytoplasm. Mutations in NPM1 are the most prevalent genetic alteration in AML, occurring in approximately one-third of cases and leading to the protein's mislocalization to the cytoplasm (NPM1c) through an additional nuclear export sequence (NES) at its C-terminus. Past research on NPM1c in leukemia has primarily focused on its cytoplasmic functions. However, our recent findings highlight a crucial role for NPM1c within the nucleus, where it unexpectedly binds to chromatin at key gene loci associated with self-renewal, such as HOXA/B and MEIS1, directly influencing oncogenic transcription. The key question now is why and how NPM1c binds to the chromatin. Our study focuses on NPM1c's export protein, XPO1, which was shown to have an important role in the chromatin recruitment of NPM1c and other oncoproteins. The goal of the study is to expand the knowledge of the non-canonical functions of XPO1 on chromatin in AML and to identify potential new therapeutic strategies.

0022 Patient-Derived Xenograft Models in Leukemia Research: Advancing Preclinical Studies

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Leukemia research is limited by disease complexity and challenges in therapy development. Patient-derived xenograft (PDX) models mitigate these issues by preserving crucial patient-specific features. We expanded primary leukemia cells in NSG or MISTRG mice, alongside 12 established cell lines, and performed RNA sequencing (RNAseq) on patient samples, PDX models, and cell lines. A paired patient/PDX analysis revealed preserved gene expression fidelity in PDXs, while cell lines mirrored only certain patient transcriptomic signatures. The 55 generated PDX models reflected the main pediatric AML subtypes, including acute megakaryoblastic leukemia, KMT2A-rearrangement, myeloid leukemia of Down syndrome, t(8;21), inv(16), NUP98-rearrangement, and RUNX1 mutations. These findings confirm that PDX models closely recapitulate patient biology, enabling translational studies and bridging the gap between in vitro experimentation and clinical testing.

0023 Investigating the role of GSK3β inhibition by Elraglusib in pediatric acute myeloid leukemia

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Glycogen synthase kinase-3 (GSK3) has emerged as a critical regulator of multiple cellular processes and a potential therapeutic target in various cancers, including AML. The role of GSK3 isoforms (α and β) in AML remains controversial. Gene expression analysis of a pediatric AML cohort revealed elevated GSK3 levels compared to healthy donors and distinct gene expression patterns in response to GSK inhibitors, particularly in AMKL, suggesting heightened sensitivity to GSK3 inhibition. Functional studies demonstrated that individual GSK3A/B depletion caused significant reduction in murine megakaryoblastic leukemia models but showed variable effects in human leukemia cell lines, although all models were uniformly sensitive to the GSK3B inhibitor Elraglusib. Interestingly, we observed that Elraglusib exerts GSK3B-independent effects, acting as a classical chemotherapeutic agent. Combining Elraglusib with the mitotic agent Palbociclib reversed these effects. These findings provide valuable insights into the mechanism of action and off-target effects of Elraglusib, with important implications for ongoing clinical trials and future development of GSK3-targeted therapies.

0024 Production of Universal CAR-T cells against the αβ-TCR with All-in-one Nanoblades

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Nanoblades are virus-like particles that contain a gRNA and a Cas9 protein to target a region of interest in the genome. In my project, we are making new All-in-one Nanoblades that contain a CAR RNA that is knocked into a targeted region. Our All-in-one Nanoblades are used to knock out the endogenous αβ TCR of T cells and simultaneously knock in a CAR that targets the αβ TCR. We have already produced All-in-one Nanoblades and demonstrated good efficiency of the CAR. After transducing primary T cells, we plan to sequence the integration site to verify the correct integration of our CAR DNA and to exclude the possibility of off-target sites. At the same time, we will use flow cytometry to check the presence of the endogenous αβ TCR and our CAR on the cell surface. After improving our transduction, we will analyse the surface markers of our CAR T cells to see if knocking out the αβ TCR has an effect on other proteins

expressed in the T cells. Finally, we will test the functionality of these CAR-T cells using cytotoxicity and degranulation assays. The CAR-T cells we have generated could be used as Universal CAR-T cells, as they should have low fratricide and no GvH-reaction.

0025 Optimizing Antibody Production for Neuroblastoma Using a Hollow-Fiber Bioreactor

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Neuroblastoma, the most common extracranial solid tumor in children, remains a major clinical challenge. Treatment strategies encompass chemotherapy, radiotherapy, surgical resection, and immunotherapy. The still poor prognosis for high-risk patients highlights the demand for novel therapeutic approaches. However, preclinical research is often constrained by the substantial antibody quantities required for in-vitro and in-vivo studies. Here, we show an optimized approach to enhance antibody production using a hollow-fiber bioreactor (HFB). A stable HEK293T cell line was generated using lentiviral transduction and was cultivated in the HFB. Cell health and antibody production were monitored across two HFB cartridges with differing oxygenation capacities and compared to conventional flask culture. Antibody yield, stability and functionality was assessed under diverse purification protocols and storage conditions. Our findings reveal a time-efficient system that maximizes antibody output while minimizing resource consumption, offering a valuable tool for advancing preclinical testing of immunotherapies.

0026 Glyco-binding domain chimeric antigen receptors as a new option for cancer immunotherapy

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Adoptive immunotherapy with chimeric antigen receptors (CARs) is a promising cancer treatment. CARs combine an antibody fragment recognizing cancer cells with intracellular domains triggering cytotoxic activity. However, suitable antigens or monoclonal antibodies are often lacking. Our study explores alternative ligand-receptor interactions beyond antigen-antibody binding. Since cancer cells show altered glycosylation, we investigated the glyco-profile of AML cells and patient samples using recombinant human lectin domains. Detection of glycan ligands in healthy tissue sections was performed, revealing the distribution and expression patterns to assess their potential relevance regarding toxic side effects. Based on these findings, we developed CAR constructs targeting AML and demonstrated specific cytotoxicity. We also tested a SynNotch system to enhance specificity and safety through a two-step T cell activation. Our novel CAR approach offers advantages over traditional CARs, including greater versatility, faster development, and reduced immunogenicity, expanding treatment options for cancers lacking antibody-based CAR targets.

0027 Targeting the HSP90-CDK9-Axis Sensitizes Pediatric AML (pAML) cells to Venetoclax

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Pediatric acute myeloid leukemia (pAML) constitutes ~20 % of leukemia cases, with relapsed disease linked to poor prognosis. Venetoclax, combined with chemotherapy or hypomethylating agents, has shown efficacy but is limited by resistance driven by upregulation of BCL-2 family members (BCL-XL, MCL-1). CDK9 inhibition suppresses MCL-1 expression by blocking RNAPII-mediated transcription, while our recent findings show that RNAPII inhibition enhances HSP90 inhibitor efficacy. Since HSP90 stabilizes BCL-2 family members, we hypothesize that co-targeting HSP90 and CDK9 could synergistically disrupt AML survival pathways. Drug screening in AML (n = 14) cell lines and (relapsed pAML patient derived) PDX-AML (n = 6) models revealed that HSP90/CDK9 co-inhibition induced a synergistic response ($p = 0.0049$) and significantly enhanced apoptosis, while sparing healthy PBMCs. Mechanistic analysis showed suppression of HSP90α, HSP70, and MCL-1 at mRNA and protein levels. Notably, dual inhibition induced apoptosis in Venetoclax-resistant AML models. These findings support HSP90/CDK9 co-targeting as a promising strategy, with ongoing preclinical evaluation of in vivo efficacy and toxicity.

0028 CRISPR-based optimization of CAR NK cell therapy for pediatric AML

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AML prognosis is significantly worsened upon relapse, and it is thus crucial to develop novel strategies for consolidation. Cellular immunotherapeutics are capable to chase even dispersed cancer cells, and with natural killer (NK) cells, they offer a potential allogeneic "off-the-shelf" therapy. However, the suitable range of effective and specific targets in AML is still limited. This project explores state of the art bulk-, long-read- and single-cell-RNA-Seq as well as proteomic data of pediatric AML patients to identify suitable immunotherapy targets. Analysis of the pediatric AML surfactome in bulk- and long-read RNA-Seq indicated the first potential targets for chimeric antigen receptor (CAR) therapy. Validation in additional datasets and single cell RNA-Seq is ongoing. In future studies, we will incorporate these targets into optimized NK cell CAR strategies with single and gated application and perform efficacy evaluation in AML cell lines as well as state of the art patient derived xenograft models for pediatric AML. Our study will provide new therapeutic venues for pediatric AML consolidation and 2nd line therapy to improve patient outcome in the future.

0029 Analyses of detailed reconstitution dynamics in Fanconi gene therapy

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Fanconi anemia comprises a group of monogenetic diseases, which all have in common that the DNA repair machinery is affected. A characteristic symptom is progressive bone marrow failure. In parallel, the impairment of DNA repair mechanisms increases the risk for the development of secondary cancers. In this project, FA will serve as a preclinical gene therapy model to investigate the prerequisites for the achievement of a stable, long-term and polyclonal hematopoiesis after stem cell transplantation (SCT) including different conditioning regimens including no conditioning. A FANCA-encoding lentiviral barcode vector enables tracking of the clonal offspring during and after repopulation. This vector will be used in FA murine SCT settings to understand how different conditioning regimens influence the competitive advantage of the corrected cells and whether this advantage leads to a therapeutic benefit. The results will directly contribute to a better understanding of the determinants that direct long-term success in gene therapeutic applications and support ongoing efforts to precisely track clonality as a continuously monitored safety feature in clinical gene therapy trials.

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0030 Development of a ROS- and lysosome-addressing combination treatment for relapsed Neuroblastoma

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High-risk Neuroblastoma (NB) patients often relapse. Mesenchymal (MES) NB are less differentiated and respond poorly to chemotherapy compared to Noradrenergic (ADR) NB. Cancer cells produce high levels of reactive oxygen species (ROS), which are involved in biological processes. At moderate levels, ROS are scavenged by antioxidants, whilst during oxidative stress cell death is promoted. Our previous data revealed a higher lysosomal content and indicated higher levels of some antioxidant-related genes in MES tumor cells. We hypothesize that more lysosomes are associated with ROS detoxification, rendering MES tumor cells chemo-resistant. Here, we aim to overcome resistance by identifying drug combinations of ROS inducers sensitizing MES NB cells, and unraveling the mechanism of interaction between lysosomes and ROS. The drug combinations will be validated ex vivo (INFORM registry-derived cultures) and in vivo (zebrafish embryo PDX models). We are currently establishing high-content imaging pipelines to study ROS and lipid peroxidation levels in multiple NB cell lines and patient-derived long-term cultures, established from fresh tissue samples, obtained through the INFORM program.

0031 Clinical evaluation of ex vivo drug sensitivity profiling in the pediatric precision oncology program INFORM

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INFORM is an international precision oncology program enrolling pediatric patients with relapsed cancer. In 2019, the program expanded its comprehensive molecular analysis to include ex vivo drug sensitivity profiling (DSP). DSP is conducted on multicellular fresh tumor spheroid cultures in 384-well plates, with an average processing time of three weeks before presentation to the INFORM board. In selected patients, notable parallels between DSP results and clinical outcomes have been observed. To systematically evaluate the predictive power of DSP, we are analyzing a cohort of 40 patients with clinical follow-up data and available DSP results. This test cohort will identify the most relevant parameter(s) for predicting clinical response. The accuracy of these predictors will be validated in a second cohort. So far, we have found that, besides classical parameters such as drug response, some other factors may be important as well, such as the number of drugs a patient has been received during one treatment regimen. Our analyses will support the development of prospective clinical trials and may eventually lead to the incorporation of DSP into personalized treatment decisions.

0032 Riboflavin metabolism as a selective therapeutic vulnerability in Acute Myeloid Leukemia (AML)

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Vitamins contribute to cellular processes, yet their role in leukemogenesis is not well defined. Here, we performed vitamin depletion screens in acute myeloid leukemia (AML) cell lines using physiological culture medium and identified vitamin B2 (riboflavin) as essential for proliferation. Using genetically diverse AML cell lines, patient-derived xenograft (PDX) models and in vivo CRISPR screens, we show that genetic depletion of riboflavin kinase (RFK), the first enzyme in riboflavin metabolism, is highly deleterious to AML. Mechanistically, loss of RFK ablated oxidative phosphorylation by impairing assembly of mitochondrial Complex I and II. The reduced respiration was marked by enhanced sensitivity to BCL-2 inhibition and recapitulated by combining BCL-2 inhibitors with roseoflavin, a riboflavin antimetabolite, showing the potential of targeting riboflavin metabolism as an anti-leukemic strategy. In solid tumor cell lines, RFK is largely dispensable. We find that expression of the gap junction channel GJA1 is a biomarker of RFK independence and hypothesize its specific expression in solid cancer cells allows circumvention of RFK by supplying downstream riboflavin metabolites.

0033 Mechanism and vulnerabilities of the SAGA complex in leukemia

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Transcriptional dysregulation drives cancer cells to become dependent on specific regulators. The SAGA co-activator complex controls transcription through histone acetylation, deubiquitylation, and splicing. Multiple SAGA complex subunits have been identified as vulnerabilities in different cancers, including pediatric leukemias. While previous studies focused on SAGA enzymatic subunits, its ~18 non-enzymatic components remain largely unexplored. To address this, we systematically analyzed all 20 SAGA subunits across six pediatric and adult ALL and AML cell lines to uncover novel vulnerabilities. Using time-resolved, SAGA-focused arrayed CRISPR screens, we identified structural SAGA CORE subunits as novel dependencies in pediatric AML. By dense mutagenesis, we identified novel critical protein domains for selected dependencies. Through pairwise AlphaFold3 predictions, we revealed potential interactions mediated by the novel domains. Finally, we could show that the depletion of SAGA CORE components destabilizes KAT2A and reduces H3K9ac levels. In conclusion, our findings support targeting non-enzymatic SAGA components in pediatric leukemia, offering new therapeutic strategies.

0034 Fine-mapping interactions of the N-terminal domain of GATA1 during leukemogenesis

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GATA1 mutations in trisomy 21 generate a truncated isoform, GATA1s, lacking the N-terminal activation domain and being pivotal to transient abnormal myelopoiesis (TAM) and myeloid leukemia in Down syndrome (ML-DS). Despite its known pathogenic role, GATA1s remains incompletely understood. Aiming to close this gap of knowledge, we will comparatively map the protein interac-

tion networks of GATA1 versus GATA1s by tagging both isoforms endogenously, followed by pull-down and proximity labeling analyses. Additionally, we employ CRISPR base editing with a targeted sgRNA library to map GATA1 exon 2 function on the amino acid level using enhanced proliferation as a surrogate for GATA1s-like transformation in fetal liver cells. This approach enriches for mutations driving a proliferative advantage. By integrating these methodologies, we aim to reveal how the absence of the N-terminal domain reshapes GATA1's protein interactions and regulatory circuits, thereby elucidating the leukemogenic mechanisms linked to trisomy 21.

0035 Targeting the fetal transcriptional landscape of pediatric AML

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Despite significant progress in the treatment of pediatric acute myeloid leukemia (AML), infant leukemia remains a clinical challenge. The presence of oncogenic events specific to infants with AML indicates a fetal origin. We hypothesized that the fetal transcriptomic landscape harbors specific vulnerabilities in infant AML. Utilizing CRISPR/Cas knock-out screens aimed at genes overexpressed in fetal hematopoietic stem and progenitor cells, we examined various cell lines and murine models. Our bioinformatic analysis revealed regulatory genes involved in pivotal cellular processes such as the cell cycle, cell proliferation and apoptosis, uncovering novel therapeutic targets across infant and pediatric AML subtypes. Selected candidates were subsequently validated in cell lines by knock-out assays. One candidate stood out in this analysis and is an interesting link between metabolism and epigenetic regulation. Ongoing research will further elucidate the contribution of this fetal gene to infant leukemia pathogenesis. This study underscores the potential of targeting fetal-origin vulnerabilities in infant AML, paving the way for innovative treatment strategies.

0036 Preemptive targeting of preleukemic cells with pathway-directed therapies: a novel approach to prevent myeloid leukemia

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Myeloid leukemia in Down syndrome (ML-DS), evolves from preleukemic transient abnormal myelopoiesis (TAM), which is marked by prenatal origins, trisomy 21, and mutations in GATA1. Our research aims to establish preemptive treatments, by targeting TAM to prevent leukemia. We employed CRISPR/Cas9- screens on a murine fetal hematopoietic stem/progenitor cell model and utilized a sgRNA library targeting genes associated with FDA-approved drugs, hypothesizing that gene knock-out can predict drug response. Integration of screening outcomes with patient derived gene expression data, essentiality scores, and pathway enrichment analyses revealed a reliance on the purine biosynthesis pathway. This led to mycophenolate mofetil (MMF), an inhibitor with minimal side effects commonly used to prevent graft vs. host disease. MMF induced apoptosis and promoted differentiation in the preleukemic TAM model, cell lines and patient-derived TAM blasts. Investigations into combination therapies revealed a synergistic effect between MMF and BCL2/BCL-XL inhibitors. Our approach identified MMF as a promising strategy to eradicate preleukemia and prevent leukemia progression in Down syndrome patients.

0037 Beneficial impact of bone marrow supportive tissues on acute lymphoblastic leukemia

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The bone marrow microenvironment (BME) facilitates leukemogenesis and therapy resistance. Studies into the relation of BCP-ALL and the BME have mostly been focused on the role of mesenchymal stromal cells (MSCs). We aim to understand which other stromal tissue types also give (prolonged) support to patients' BCP-ALL cells, to develop a representative ex vivo BME model, enabling functional studies. All tested supportive cell types provided ex vivo survival benefit to BCP-ALL cells with a median benefit of 18-24 % (chondrocytes and early adipocytes) and 30-36 % (MSCs and osteocytes). Exposure of leukemic cells to a mixture of supportive tissue types in a 3D microfluidics model induced increased cell proliferation compared with leukemic cells co-cultured with each of the individual supportive cell types separately. Our data suggest that BCP-ALL cells manipulate different components of the bone marrow supportive tissues similarly, favoring survival of leukemic cells and creating a pro-inflammatory microenvironment. Current studies are addressing the impact of a leukemic BME on the efficacy of cellular immunotherapies like blinatumomab and CAR-T.

0038 Deciphering impact of B-cell precursor acute lymphoblastic leukemia bone marrow microenvironment on immune cell function

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B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cells residing in the bone marrow modulate supportive cells to create a leukemic niche facilitating their own persistence. Elucidating the influence of leukemic niche formation on immune cells is essential to assess its impact on cell-based immunotherapies. We performed mono- and co-cultures of mesenchymal stromal cells (MSCs) and ALL cells to assess changes in gene expression upon co-culture. Total-RNA sequencing revealed an interferon a/b (IFNa/b)-related gene signature in MSCs upon co-culture with ALL compared to mono-culture, which is ETV6::RUNX1-subtype linked. However, ALL cell viability and chemotherapy resistance were not affected upon inhibition of IFN signaling. Secretome analysis revealed upregulation of pro-inflammatory cytokines, and chemokines such as CCL4, CXCL5, CXCL8, and CXCL10 in the leukemic niche. We will assess gene expression changes in other bone marrow supportive cell types upon co-culture with ALL cells. The influence of the leukemia-induced IFNa/b gene signature in bone marrow supportive cells on the response to immunotherapy, such as blinatumomab, is currently being studied.

0039 Deciphering the role of non-coding sequence variants predisposing to pediatric B-ALL

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Most trait- and disease-associated sequence variants map at non-coding regions, modulating transcription factor (TF) binding and transcription of the associated gene. Multiple genome-wide association studies linked several non-coding germline variants to increased risk of pediatric B-ALL, particularly at the IKZF1 locus. However, the consequence of intergenic variants at IKZF1 and the mechanism of disease predisposition remain largely unknown. Here, we established IKZF1-GFP reporter NALM6 and REH cell lines and a multi-scale screening approach to map the regulatory network of IKZF1, identify top regulators, and elucidate the effect of disease-associated variants at TF binding motifs. To uncover putative cis-regulatory elements, we applied a CRISPRi screen tiling ~400kb at the IKZF1 locus, including its promoter, 3' UTR, proximal and distal enhancers. In this screen, we identified two distal enhancers as well as a cis-regulatory sequence located at the IKZF1 3' UTR. In parallel, to identify trans-regulators of IKZF1, we performed a KO screen, targeting 635 TFs expressed in the hematopoietic lineage, and identified a shortlist of high-confidence IKZF1 regulators.

0040 CRISPR-Cas-Based Optimization of (CAR-)NK Cell Therapy in Pediatric AML

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NK cell immunotherapy is a highly promising approach for pediatric AML. Here we aim to enhance efficiency by identifying and targeting immune-evasive pathways via drug repurposing. To this end, a CRISPR-Cas9-based high-throughput screening targeting 1150 gene-drug interactions will be utilized to uncover druggable modulators of NK ligand expression to boost cytotoxicity. First, pediatric AML patients were characterized for NK cell ligand expression (RNA-Seq). Patients revealed variable dysregulation of activating ligands (e.g. TNFSF9, BAG6, MICA, NID1), a moderate expression of killing receptors (e.g. TRAIL-R2, FAS) and coreceptors (e.g. Nectin-2, PVR), while the inhibitory HMGB1 is highly expressed in most AML subtypes. Next, we will validate these findings on protein level via flow cytometry (FC), followed by FC-based CRISPR screening for possible drug targets modulating receptor expression. After validating our screening, AML cells (knock-out or drug treated) will be co-cultured with (CAR-)NK cells to assess NK cell sensitivity or resistance. The ongoing study will identify targetable pathways to enhance NK cell efficiency for the development of combination therapies.

0041 Generation of iPSC-Derived Mesenchymal Stromal Cells (iMSCs) for Ex Vivo Acute Myeloid Leukemia (AML) Niche Models

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The development of AML depends on cell-extrinsic changes from the bone marrow (BM) microenvironment modulating leukeamia initiation and progression. However, studying the communication between AML cells and niche components requires reliable and modifiable niche components. Here, we describe the controlled differentiation of induced pluripotent stem cells (iPSC) into iMSC lines through mesodermal and ectodermal induction pathways. Five distinct iMSC lineages were generated and thoroughly characterised throughout their production process. Their stemness and stability were assessed using tri-lineage differentiation and colony formation assays revealing differences in their pre-osteoblastic or pre-adiposite potential. Notably, these iMSC lineages exhibit varying capacities to support the maintenance of immature primary AML cells in iMSCs/AML co-culture systems. Together, we describe the generation of distinct iMSC lines that mimic key AML supporting components within the BM niche. These iMSC will serve as a tool for the development of BM-like platforms enabling the investigation and controlled manipulation of the crosstalk between AML cells and the stromal microenvironment ex vivo.

0042 Molecular and histological analyses of AT/RT-TYR suggest the choroid plexus of the fourth ventricle as cellular origin

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Atypical teratoid/rhabdoid tumors (AT/RT) are the most common malignant brain tumors during infancy. They are characterized by SMARCB1 or SMARCA4 mutations and split into the four molecular groups AT/RT-TYR, AT/RT-SHH, AT/RT-MYC, and AT/RT-SMARCA4. Each of these types has distinct clinical characteristics, with AT/RT-TYR most frequently occurring in the fourth ventricle. However, details on tumor initiation as well as the cellular origins remain largely unknown. Notably, mouse models for AT/RT-TYR providing insights into tumor development and prerequisites to explore treatment strategies are completely lacking. Here, we show that AT/RT-TYR very often appear intermingled with fourth ventricle choroid plexus (CP) tissue and that tumor cells heavily express CP markers. Analyses of bulk- and single-cell RNA sequencing data across various brain tumor entities and cell populations of the developing normal brain reveal a clear resemblance of the AT/RT-TYR to the CP of the fourth ventricle. Finally, Foxj1-cre::Smarcb1fl/fl mice showing loss of Smarcb1 in early CP progenitors gave rise to large atypical CP cells resembling rhabdoid cells with gene expression most similar to human AT/RT-TYR.

0043 Inter- and intratumoral heterogeneity in MYCN-amplified spinal ependymoma.

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MYCN-amplified spinal ependymoma (SP-EPN-MYCN) represents a novel high-risk CNS tumour and no established therapeutic protocols. Here, we applied a multi-omics approach to investigate the molecular mechanisms in SP-EPN-MYCN. Copy number variation (CNV) analysis in a cohort of 111 samples (n = 76 patients, 35 relapses) showed an amplification of MYCN in 108 and MYC in three tumours. Other CNVs included a loss of chromosome (Chr.) 10 (35/111). Single nucleus RNA-sequencing in 13 cases (25914 nuclei) revealed a diverse tumour microenvironment with both pro- and anti-inflammatory types of monocytic cells as well as tumour-infiltrating lymphocytes. The malignant cells showed upregulation of several biological programmes, including MYCN-driven proliferation, astrocytic and ependymal markers as well as neuronal signalling, pointing towards a glial progenitor of SP-EPN-MYCN.

0044 Epigenetic maintenance of oncofetal identity in infant AML

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The maintenance of oncofetal gene expression is a hallmark of infant AML, yet the epigenetic regulation driving this aberrant program remains unclear. To elucidate these networks, we will perform unbiased CROP-Seq alongside a focused analysis of PRC2 and SWI/SNF, two opposing epigenetic complexes in hematopoietic development. Using CRISPR-based knock-ins, we will fluorescently label the catalytic subunits EZH2 and SMARCA2/4, enabling flow cytometry-based CRISPR screens to identify regulatory mechanisms at post-transcriptional and post-translational levels. By integrating the analysis with IP-MS and CUT&RUN, we will dissect the composition and deposition of these complexes, constructing a comprehensive map of their roles in infant AML. Ultimately, this work will reveal how oncofetal epigenetic regulation is shaped and maintained, particularly by PRC2 and SWI/SNF, to drive AML pathogenesis, and will guide the development of epigenetic therapies that target the oncofetal state.

0045 Exploring the translational potential of EZH2-controlled fetal gene signature in AML

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Enhancer of zeste homolog 2 (EZH2) is the main catalytic subunit of PRC2, guiding H3K27 tri-methylation and gene silencing. Mutations in EZH2 occur in 2–13 % of AML patients. Our work showed that EZH2 loss reactivates fetal gene signatures, typically silent in adult cells but elevated during embryogenesis and tumorigenesis. Such reactivation, enriched in patients with EZH2 or PRC2 mutations, emerged as a leukemogenic driver in mouse models and was predominantly observed in high-risk AML patients. To probe these fetal gene signature we performed CRISPR-Cas9 screens to pinpoint reactivated fetal genes that function as therapeutically exploitable vulnerabilities. Additionally, we tested and optimized mutant Cas12a based CRISPR interference to complement the targeting of coding variants with fine mapping enhancers that regulate fetal gene expression. By illuminating the molecular mechanisms of EZH2 in driving fetal gene reactivation, our study will advance targeted therapeutic strategies for AML patients.

0046 Deciphering the role of KANSL1 mutations in the development of Myeloid Leukemia in children with Down Syndrome (ML-DS)

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DOI 10.1055/s-0045-1808998

Transient abnormal myelopoiesis (TAM) is a form of clonal hematopoiesis seen in children with trisomy 21 caused by mutations in the transcription factor GATA1. TAM clonally progresses to myeloid leukemia in Down syndrome (ML-DS) upon acquisition of secondary mutations. Utilizing a virus-free CRISPR screening platform, GATA1 and additional mutations were introduced into primary human fetal liver hematopoietic stem and progenitor cells. In vitro and in vivo xenotransplantation assays revealed KANSL1 mutations to be a potent oncogenic driver of the progression from TAM to fully developed leukemia. To characterize the role of KANSL1 mutations, we performed loss-of-function assays revealing domains essential for normal and malignant hematopoiesis. Additionally, we aim to describe KANSL1 interactome to understand its contribution to the epigenome and gene expression. We are conducting proteomic, transcriptomic and epigenomic assays alongside the generation of KANSL1-tagged cell lines with a degradation tag. This will help define the impact of mutated KANSL1 on downstream pathways. Future research will explore new therapeutic vulnerabilities in ML-DS patients carrying mutated KANSL1.

0047 Targeting novel chromatin vulnerabilities in childhood acute myeloid leukemia

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Although survival rates in pediatric AML (pAML) increased, a deeper understanding of epigenetic oncogene regulation through long non-coding RNAs opens avenues for novel treatments. In our study, we explore the AML transcriptome landscape by comparing RNA sequencing of pediatric AML patients to healthy individuals. We identified 696 differentially expressed long non-coding RNAs (lncRNAs), implying a potential function in pAML . Utilizing CRISPR/Cas9 inhibition we probed these lncRNAs for function and identified non-coding elements essential in AML development. Validation through knockdown assays followed by quantitative PCR (qPCR) in AML cell lines and patient-derived xenografts (PDX) helped us determine a novel chromatin vulnerability and its relation to oncogene expression. Further analysis with target perturbation followed by a multi-omics approach on transcriptome and chromatin accessibility underscored the critical role of chromatin-mediated oncogene regulation in pediatric AML. Our investigations open new pathways for therapeutic development emphasizing the latent potential of targeting epigenetic mechanisms in combatting this malignant disease.

0048 Deciphering the interplay between DNA methylation and CTCF in AML

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DNA methylation is often dysregulated in acute myeloid leukemia. Aberrant CpG methylation contributes to malignancy by influencing transcription directly but potentially also indirectly through differential binding of the transcription factor CTCF which mostly occupies hypomethylated DNA. Here, we profile methylation status, CTCF occupancy and gene expression during perturbative experiments in which CTCF is degraded using an auxin-inducible degron or DNA hypomethylation is obtained using azacitidine. By systematic investigation of the interplay between different epigenomic layers at genes and CpGs that are associated with prognosis and drug sensitivity, we aim to gain insights into how the epigenome contributes to leukemogenesis and therapy response.

0049 Memory-like NK cell and CD19-antibody based immunotherapy combined with TKI has antitumor effects against Ph(-like) ALL

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Philadelphia-like acute lymphoblastic leukemia (Ph-like ALL) is a molecularly distinct tyrosine kinase-driven entity burdened with a high risk of relapse and

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poor response toward combinatorial chemotherapy. Tyrosine kinase inhibitors (TKI) have been in clinical use to improve the survival of patients with Ph-like ALL, yet preliminary data indicate subpar outcome. To advance treatment concepts engineered antibody and cell-based immunotherapy has been proposed. Allogeneic memory-like natural killer (ML-NK) cells had been used to treat leukemia with a low risk of graft-versus-host reaction. Previously, we demonstrated that optimized ML-NK cell and CD19 antibody-based immunotherapy combined with selective TKI demonstrates significant in vitro treatment efficacy in kinase-driven leukemia models. Lately, we investigated the in vivo treatment efficacy of ML-NK cell and CD19 antibody-based immunotherapy combined with TKI against patient-derived xenografted Ph-like ALL. Our proposed treatment approach was associated with, lower tumor burden, ML-NK cell persistence and increased leukemia-free survival compared to TKI monotherapy.

0050 Development of an Imaging-Based High-Throughput Platform for Ex Vivo Agent Treatment in Patient-Derived AML Cells

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Patient-derived AML cells offer a more personalized platform for drug screening, yet their ex vivo culture is limited by the rapid loss of proliferative capacity, unwanted differentiation and low viability. We aim to develop an image-based personalized drug screening platform using our AML/mesenchymal stroma cell (MSC) co-culture system. First, we optimized our AML/MSC co-culture system for high-throughput compound testing in a 384-well format. To assess the effects of compounds on both, AML cells and MSC, we performed CyQuant staining of viable cells, followed by confocal high-content imaging. The acquired images were analyzed using a deep learning model for cell segmentation and classification into AML or MSC populations. A U-Net structured model was trained using a versatile dataset, including images from cell lines, patient-derived xenografts, and primary AMLs. The model achieved an AML-MSCs classification accuracy of 0.97, which was further validated by manual cell counting. Together, we developed a machine learning approach for high-throughput AML drug screening, quantifying true cell numbers. Future work will enhance robustness and explore differentiation state detection.

0051 CD127-directed immunotherapy outperforms Imatinib in preclinical ABL-class fusion + BCP-ALL via a dual mode of action

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ABL-class fusion-positive (ABL-fusion +) B-cell precursor (BCP-)ALL is treated with chemotherapy and tyrosine kinase inhibitors such as Imatinib (Ima), a combination that can cause severe toxicity. We recently showed potent preclinical efficacy of CD127-directed immunotherapy (CD127-IT) in ALL. Hence, we preclinically evaluated CD127-IT in combination with Ima in ABL-fusion + BCP-ALL. Ima enhanced CD127 expression in ABL-class fusion + cell lines and patient-derived xenograft (PDX) samples. Both Ima and CD127-IT induced macrophage-mediated phagocytosis as single agents, which was enhanced in combination. Ima and CD127-IT abrogated BCR-ABL1- and IL-7-mediated STAT5 induction, with strongest impact on the pathway upon combi-treatment. In a phase II-like PDX-trial, CD127-IT was effective in 7/7 PDX-samples in vivo and outperformed the effect of Ima alone. CD127-IT was more effective when combined with Ima as compared to CD127-IT alone. Mechanistically, downregulation of the “don’t eat me” signal CD47 could be demonstrated by RNA sequencing analyses. Our data shows preclinical efficacy of CD127-IT plus Ima via phagocytosis induction and CD127-signaling blockade in ABL-fusion + ALL.

0052 Investigating Senescence-Induced Tumor Plasticity in High Risk Neuroblastoma

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Senescence promotes tumor cell plasticity and intratumor heterogeneity, with post-senescent cells contributing to aggressive relapses. Therefore, this project investigates the role of therapy-induced senescence (TIS) in tumor plasticity in high-risk MYCN-amplified (MNA) and non-MYCN-amplified (non-MNA) neuroblastomas to identify pathways of treatment resistance. In cell lines and patient-derived xenografts (PDX) both cytotoxic and targeted therapies induced TIS in MNA and non-MNA neuroblastomas, as demonstrated by proliferation assays, SA-ß-gal activity, and H3K9me3 immunofluorescence. Since both subgroups eventually escaped TIS, post-senescent populations were isolated via fluorescence-activated cell sorting and rechallenged with cisplatin to evaluate newfound resistance mechanisms. Both untreated and post-senescent tumor cells -/ + cisplatin, as well as untreated and therapy-exposed PDX, were analyzed by label-free proteomics to identify signaling pathways and molecular phenotypes driving senescence-induced plasticity and resistance. Hereby we aim to uncover targetable vulnerabilities in post-senescent neuroblastoma cells to prevent relapses and improve treatment outcome.

0053 TMZ Sensitization in GBM Cell Lines by Sequential Fadraciclib Treatment: An MGMT-Independent Strategy

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Current therapies for glioblastoma (GBM) results in a median survival of 15 months, underscoring the need for improved strategies. Since cyclin-dependent kinases (CDKs) regulate the cell cycle and contribute to carcinogenesis, we investigated the CDK2/9 inhibitor, fadraciclib, in combination with temozolomide (TMZ) in tumor spheroid models.
Spheroids from four patient-derived GBM cell lines (GBM03, 06, 14, and 15) were treated with fadraciclib (IC50) and TMZ (10 mM) in mono- and combination therapy in a sequential approach (with TMZ administered following CDK blockade). Viability and cytotoxicity were measured using CellTiter-3D Glo® and LDH-Glo™ assays. The Bliss independence index identified the most effective combination for invasion analysis. All four cell lines demonstrated sensitivity to fadraciclib, the methylated GBM06 cell line showed the expected higher sensitivity to TMZ compared to the unmethylated ones. The addition of fadraciclib synergistically enhanced the antitumoral effects, particularly in the unmethylated cell lines. Sequential combination therapy induced a synergistic effect, significantly reducing viability, increasing cytotoxicity, and inhibiting invasion.

0054 High-throughput drug screening of triple combinations in pediatric acute myeloid leukemia

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Pediatric AML is genetically and morphologically heterogeneous. Although intensive chemotherapy has improved outcomes, high-risk patients remain vulnerable to relapse and face significant toxicities. While targeted combination therapies may enhance both prognosis and safety, large-scale discovery of synergistic treatments is challenging. We propose a surrogate multiplex drug screening approach using RNA-targeting CRISPR-Cas13. After validating Cas13d stability and guide efficiency via a prescreen of over 300 drug targets, we concurrently target triple drug–gene interactions by silencing relevant mRNAs, enabling identification of synergistic target combinations. A three-step screening in AML cell lines precedes in vivo testing in preclinical AML PDX models and subsequent validation with small-molecule inhibitors. This system will yield new insights into pediatric AML heterogeneity and illuminate potential synergistic therapeutic vulnerabilities.

0055 Extrachromosomal DNA as a potential therapy target in a pediatric high-grade glioma mouse model with MYCN amplification

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Pediatric high-grade gliomas (pHGG) are rare, molecularly heterogeneous tumors with 3 distinct subtypes identified through global DNA methylation profiling. The most aggressive and prevalent subtype, pHGG-MYCN, typically arises in children at a median age of 8 and is frequently characterized by MYCN amplification. It resists current treatments, resulting in a median overall survival of just 14 months. To search for alternative therapeutic targets, we performed single-cell RNA sequencing on tumors from a pHGG-MYCN mouse model, revealing heterogeneous MYCN expression and focal transgene amplification in all tumors. These results suggest oncogene variability linked to extrachromosomal DNA (ecDNA), a known driver of tumor growth and heterogeneity in pediatric cancers. To investigate ecDNA in our mouse model, we performed whole-genome sequencing on 8 tumors, detecting integration site-containing amplicons in all samples using the AmpliconSuite pipeline. Further classification revealed ecDNA-like structures in 6 out of 8 cases. Follow-up validation aims to confirm ecDNA involvement in both mouse and human tumors, highlighting it as a promising target for focalized therapy in pHGG-MYCN.

0056 ctDNA from preoperatively collected CSF predicts molecular classification of brain tumors

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The precise molecular diagnosis of central nervous system (CNS) tumors significantly impacts tumor resection and cancer treatment. While the current standard for tumor classification relies on the analysis of surgically obtained tumor tissue, liquid biopsy has emerged as a promising alternative, allowing for the preoperative detection of tumor-derived biomarkers from minimally invasively collected body fluids. In this study, we analyzed n = 58 cerebrospinal fluid (CSF) samples collected preoperatively from patients suspected of having CNS tumors. Cell-free DNA was analyzed by nanopore sequencing focusing on copy number variations (CNVs) and DNA methylation profiles using a neural network-based tumor classification approach. Presence or absence of circulating tumor DNA (ctDNA) was correctly determined in 48.3 % of cases, as proven by subsequent tissue biopsy. In addition to specific CNVs present in the cases with detectable ctDNA, tumor classification was possible based on global DNA methylation in 37.5 % of these cases with all of them delivering the correct tumor type. In the remaining 51.7 %, ctDNA was undetectable despite a neuropathologically confirmed CNS tumor diagnosis.

0057 Exploring the local tumor infiltration of medulloblastoma into the adjacent brain tissue

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Medulloblastoma (MB), one of the most common malignant brain tumors in children, is classified into four molecular types: Sonic Hedgehog (SHH), WNT, and non-WNT/non-SHH (group 3 and group 4). While subtype-specific metastasis patterns have been described, and metastasis at diagnosis is a negative prognostic factor for patient survival, local infiltration of MB into the surrounding brain tissue remains largely unexplored. This project aims at investigating the local infiltration of primary MB. By examining hematoxylin and eosin-stained sections from over 250 MB, we observed that local infiltration into the cerebellar tissue was more frequently displayed in SHH than WNT or non-WNT/non-SHH MB. Using single-nucleus RNA sequencing data, a various number of non-neoplastic cerebellar granule cells was detected in 17 of 93 MB samples (SHH, n = 10; group 4, n = 6; and WNT, n = 1), with the presence of granule cells correlating with the histopathological evaluation. Ongoing efforts involve the generation of spatial transcriptomic data, assessing brain infiltration on MRI, and correlating our findings with clinical data to deepen our knowledge on the local tumor infiltration in MB.

0058 Mesenchymal Stromal Cells do not suppress CAR-T function in B-Cell Precursor Acute Lymphoblastic Leukemia

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DOI 10.1055/s-0045-1809010

CD19-directed CAR T-cell therapy for pediatric relapsed/refractory B-cell precursor acute lymphoblastic leukemia (BCP-ALL) revealed promising short-term outcome results. However, still 50 % of these cases relapse within 2 years after CAR T-cell infusion. Our previous research showed that BCP-ALL hijack the bone marrow niche resulting in increased leukemic cell survival, chemotherapy resistance and immunosuppression. Our aim is to investigate whether MSCs in the bone marrow niche impact the killing efficacy of CAR T-cells. Ex vivo CAR-T efficacy assays of 10 diagnostic BCP-ALL bone marrow aspirates were performed by coculturing BCP-ALL cells with MSCs for 40h, mimicking the ALL-educated niche ex vivo. This was followed by exposure to CAR T-cells for 24h and flow cytometry to assess survival of BCP-ALL cells. For 80 % (8/10) of the samples, there was no difference or even more CAR T-mediated killing seen in coculture with MSCs compared to monoculture, showing that MSCs do not protect BCP-ALL cells against CAR T-mediated killing in this in vitro setting. Ongoing experiments will show whether CAR-T function is affected by coculture with other differentiated stromal layers.

0059 Development and validation of an ELISA for the quantification of anti-ALK autoantibodies in ALCL Patients

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ALK-positive Anaplastic Large Cell Lymphoma (ALK + ALCL) are characterized by genetic rearrangements involving the ALK gene with expression of ALK fusion proteins. 90 % of pediatric ALK + ALCL patients harbour the (2;5) translocation that leads to the expression of the nucleophosmin anaplastic lymphoma kinase oncoprotein. ALK-specific autoantibodies are detected in the plasma of 90 % of pediatric ALK + ALCL patients at diagnosis, and the strength of the ALK-specific antibody response is inversely correlated with relapse risk. Currently, ALK autoantibody titres are measured by an indirect immunoperoxidase assay using cytospin preparations of ALK-transfected COS-1 cells for microscopic assessment. However, this method is time-consuming, requires subjective microscopic evaluation, and lacks a quantitative approach, which prohibits using the method in clinical studies or routine care. Therefore, we are developing and validating an indirect enzyme-linked immunosorbent assay (ELISA) to quantify ALK autoantibodies. The sensitivity, specificity, inter- and intra-assay variances are currently under investigation. A comparison of both methods with patient plasma will be performed and presented.

0060 Enhancing Nanopore cfDNA diagnostics of pediatric brain tumors with machine learning

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Algorithmic methods are redefining diagnostic workflows for brain tumors. Various pipelines for molecular diagnoses based on DNA from solid tumor tissue are used in clinical practice today. While methylation arrays remain the gold standard for methylation profiling, Nanopore sequencing has become a promising method, facilitating overnight diagnostics. Recent works have shown that it can further enable both methylation- and CNV-calling from cerebrospinal fluid (CSF) cell-free DNA (cfDNA), allowing for minimally-invasive tumor diagnoses. However, there are currently no specific classification algorithms tailored to this task. We trained a classification algorithm on 1,432 publicly available methylation profiles of a selection of 16 pediatric tumor entities and control tissues. To tune and evaluate its performance, we assembled a cohort of 67 pediatric CSF samples with a confirmed tumor diagnosis and a known presence of cfDNA. Our model scores a balanced accuracy of 67 % on the test set, outperforming the currently used published models. This promising result highlights the potential of our approach in improving diagnostic accuracy for pediatric brain tumors.

0061 Multi-functional potency assay for CAR-NK cells: Mapping cytotoxicity, degranulation and proliferation in one

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The number of clinical trials worldwide using CAR-T cells and recently also CAR-NK cells against diverse hematological targets is growing steadily. To safely apply CAR-NK cell products, GMP-compliant manufacturing and a validated Quality Control (QC) is crucial. Regulatory requirements for QC of ATMPs are extensive yet it must be practical and feasible in terms of time and manpower commitment. As part of QC, we therefore developed a flow-based multi-functional potency assay for (CAR)-NK cell products, that determines various test parameters at once. Most flow cytometric cytotoxicity assays primarily stain target cells. However, these dyes are either cytotoxic at higher concentrations or do not bind permanently, making them unsuitable for staining effector cells. Instead, VPD is used as an alternative dye, which binds irreversibly to intracellular proteins in NK cells without being cytotoxic. With this new approach, effector cells can be stained and tracked long-term. This offers the advantage of not only visualizing NK cell cytotoxicity but also extending the functional assay into a broad potency assay mapping also fitness, degranulation and proliferation in a single approach.

0062 miRNA signatures in Burkitt leukemia and lymphoma in children and young adults

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Data on miRNA expression profiles in Burkitt leukemia (B-AL) and Burkitt lymphoma (B-Ly) are scarce. The aim of this study was to evaluate differential miRNA expression in a series of pediatric and young adult B-AL/B-Ly to identify miRNA signatures associated with clinicopathological features and outcome. We evaluated the expression profiles of 800 miRNAs in 33 B-AL/B-Ly using the NanoString nCounter System. Further validation was performed by qPCR using miRNA-specific TaqMan assays. A miRNA-mRNA network analysis was performed in silico to explore experimentally validated target genes and involved signaling pathways. Differential miRNA expression analysis when comparing B-AL to B-Ly identified sets of significantly overexpressed miRNAs (miR-223-3p, miR-451a, miR-150-5p, miR-144-3p, miR-142-3p and miR-15a-5p) and lower expressed miRNAs (miR-494-3p, miR-4286, miR-1915-3p, miR-125b-5p and miR-100-5p) (> + /-5-fold change (FC), p-adj < 0.01). Notably, significant downregulation of miR-10a-5p (-1.97-FC, p-adj 0.01) was observed in the unfavorable outcome group. Novel miRNA signatures significantly associated with leukemic presentation, age and outcome could be recognized.

0063 CCND3 Knockout Impairs Leukemia Growth in PDX AML Models Revealing a New Therapeutic Vulnerability

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DOI 10.1055/s-0045-1809015

Acute myeloid leukemia (AML) is a highly heterogeneous malignancy with a poor prognosis due to high relapse rates and limited therapeutic options. Current treatments exhibit restricted efficacy because of intrinsic resistance mechanisms and adverse side effects. These challenges highlight the need to identify context-dependent, targeted novel therapies. In this study, using genetically engineered patient-derived xenograft (PDX) models, we aimed to discover dependency genes in AML that could serve as potential therapeutic targets. Utilizing the Cancer Dependency Map, we designed a customized CRISPR library targeting 49 genes essential for AML. We screened this library across 3 PDX models; from one pediatric and two adult patients and identified CCND3 as one of the top dropout genes in all AML samples. To validate CCND3 as a

dependency gene, we performed an in vivo competitive growth assay, transplanting a 1:1 mixture of control and CCND3-KO AML cells. The assay confirmed that CCND3 is critical for AML cell survival as control cells outcompeted CCND3-KO cells, which failed to grow. These findings suggest that CCND3 is a novel dependency gene and a potential therapeutic vulnerability.

0064 Analysis of novel transcripts and alternative isoform usage in acute myeloid leukemia

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Rapid development of sequencing techniques has allowed for more accurate genome annotations, especially for the human genome. We have recently discovered that, at bidirectional promoters, the transcription factor CTCF represses transcription initiation of upstream, antisense transcripts, many of which were not annotated before. Using the meta-assembly tool Aletsch, we built an optimized pipeline to explore known and unknown transcripts in both adult and pediatric leukemia. We managed to discover novel transcripts and isoforms in patient samples which we predict to be related to leukemogenesis. A subset of those novel transcripts has protein coding potential and might thus encode microproteins. By integrating other omics techniques, such as Ribo-seq and Proteomics, we aim to further characterize these putative ORFs. Our pipeline does not only allow for a better comprehension of the leukemia transcriptome, but also for the discovery of biomarkers as well as potential targets for immunotherapy

0065 Understanding the role of linear ubiquitination in lysophagy and lysosome-mediated cell death in acute myeloid leukemia

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AML is an aggressive blood cancer with high primary refractory and relapse rates and urgent needs for new treatments. Lysosomes are essential in cellular recycling, maintaining homeostasis, and buffering of chemotherapeutic drugs. AML cells exhibit larger, but more fragile, lysosomes and display increased expression of lysosomal biogenesis genes. Because of this, lysosomotrophic agents that disrupt lysosomal membranes and induce lysosomal cell death may selectively target therapy resistant AML. Damaged lysosomes are ubiquitinated to recruit the autophagy machinery for autophagic degradation called lysophagy, but the specific role of linear ubiquitination and the linear ubiquitin-specific deubiquitinating enzyme OTULIN in lysophagy remains unknown. Here we show that OTULIN depletion enhances linear ubiquitin levels, increased AMPKα phosphorylation and LC3B accumulation in AML cells. Furthermore, induction of LMP triggers OTULIN-dependent cell death in a selected set of AML cells. Our results demonstrate regulation of autophagy by linear ubiquitination in AML.

0066 Spatial transcriptomics in paediatric and adult acute myeloid leukaemia: unravelling the bone marrow microenvironment

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Acute myeloid leukaemia (AML) urgently requires novel therapeutic strategies. However, immunotherapy has so far shown limited results, and the cellular interactions driving immune evasion remain poorly characterized. To address this gap, we applied Xenium imaging-based spatial transcriptomics to 62 formalin-fixed paraffin-embedded bone marrow (BM) biopsies from AML patients (26 children, 18 adults) and non-leukemic controls (9 children, 9 adults), yielding 474,580 high-quality single-cell spatial transcriptomes. This enabled accurate characterization of the BM composition in situ, identifying a substantially higher frequency of stromal cells (median 5 %, range 2-19 %) and macrophages (3 %, 0.3-21 %) compared to typical BM aspirate-based analyses. Importantly, fusion probes allowed identification of cells carrying the RUNX1::RUNX1T1 fusion, and spatial analysis revealed a distinct organization of cellular niches in the healthy BM, which is lost in AML. The insights generated by this approach are of great value to characterize cell-cell interactions related to immune evasion and to identify potential therapeutic targets, paving the way for successful immunotherapy in AML.

0067 Glycosylation and Immune Evasion in Neuroblastoma

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Neuroblastoma (NB) is the most common extracranial solid tumor in children, with high-risk cases exhibiting poor prognosis despite intensive multimodal treatment. The tumor's genetic and molecular heterogeneity complicates therapy, underscoring the need for novel therapeutic strategies. Our study investigates key regulatory mechanisms in NB, particularly the role of glycosyltransferases in immune evasion and tumor progression. Dysregulated glycosylation patterns alter immune recognition and impact NB aggressiveness, providing potential targets for intervention. Our findings contribute to the understanding of NB pathophysiology and highlight glycosylation as a promising avenue for therapeutic exploration. Targeting glycosylation-related mechanisms may enhance treatment efficacy and improve patient outcomes.

0068 In vitro and in vivo comparison of VHH- and scFv-based CLEC12A-chimeric antigen receptor-natural killer cells against acute myeloid leukemia

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Treatment of acute myeloid leukemia (AML) remains challenging due to its heterogeneity and lack of suitable target antigens. CLEC12A (CLL-1), expressed on leukemic blasts and leukemia-initiating cells in up to 92 % of AML patients, offers an attractive target for CAR-based immune cell therapy. CAR-engineered natural killer (NK) cells represent a viable off-the-shelf treatment option for hematological malignancies, combining targeted cytotoxicity and innate immune responses with a low risk of graft-versus-host disease. To enhance anti-leukemic activity, we developed a CLEC12A-CAR-NK cell product featuring a novel VHH binding domain identified via llama immunization and yeast surface display. Combined with a 4-1BB-CD3ζ signaling domain and IL-15 armoring as CAR construct, these VHH-CAR-NK cells showed enhanced in vitro cytotoxicity compared to scFv-based CAR-NK cells and non-transduced NK cells. Cytotoxic efficiency was especially promising at low effector-to-target ratios. In an OCI-AML2 xenograft model, VHH-CAR-NK cells reduced tumor burden and

increased survival more efficiently than the scFv-based counterpart, demonstrating their potential as effective AML-targeting cell therapy.

0069 Epigenetic remodeling via HDAC6 inhibition amplifies T cell responses in pediatric myeloid lineage leukemia

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HDAC6 has emerged as a promising therapeutic target in cancer due to its immunomodulatory function. While its prognostic role remains debated, we showed that HDAC6 loss impairs myeloid leukemia progression in vivo without affecting in vitro proliferation. Proteomics analysis of HDAC6-KO cells revealed upregulation of lysosome-associated proteins (LAMP1) and innate immune-related pathways, including RNaseT2, a tumor suppressor known to modulate the tumor microenvironment. This effect was specific to myeloid leukemia and absent in B-ALL. Pharmacological HDAC6 inhibition with Ricolinostat not only induced RNaseT2 upregulation in myeloid leukemia cells, but also increased chromatin accessibility of RNaseT2 and LAMP1, highlighting an epigenetic regulatory mechanism. Further, Ricolinostat sensitized murine AML cells to CD8+ T cell cytotoxicity via increased TNFα secretion ex vivo and in vivo. A high-throughput drug screen revealed strong synergy between Ricolinostat and Cytarabine or Clofarabine, with minimal effect on ALL or healthy controls identified. Our study highlights HDAC6 inhibition as a dual-modality strategy in myeloid leukemia, boosting immune activation and Chemosensitivity.

0070 Unveiling the Molecular Complexity of AML through Advanced Multi-Omics Analysis

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The HemAtlas 2.0 project embarks on a groundbreaking multi-omics journey to unravel the complexities of pediatric acute myeloid leukemia (AML), incorporating a diverse range of pediatric cases including transient myeloproliferative disorder (TMD), myeloid leukemia of down syndrome (ML-DS) and AML defined by fusion oncogenes. Utilizing advanced techniques such as WES, RNA-seq, and ATAC-seq, aiming to decode AML's genetic and epigenetic complexity. Integrating multilateral data with clinical insights, our work advances AML subtype classification and elucidates the impact of somatic mutations on prognosis, particularly affecting overall and event-free survival. Through Multi-Omics Factor Analysis (MOFA), we uncover intricate molecular interactions that define AML's heterogeneity. This novel approach provides deep insights into AML's pathogenesis and identifies potential targets for therapy. Our findings underscore the benefits of multi-omics integration in enhancing disease understanding to improve classification and prognosis. The HemAtlas 2.0 project highlights the potential of combining diverse omic layers and clinical data to refine patient care strategies.

0071 Understanding the role of linear ubiquitin in lysophagy and lysosomal cell death in diffuse large B-cell lymphoma

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Aberrant NF-κB activation is a hallmark of activated B-cell-like diffuse large B-cell lymphoma (ABC-DLBCL) and underlies proliferation, survival and inflammation. NF-κB is driven by linear (M1) poly-ubiquitin (poly-Ub) chains, catalysed by the M1 poly-Ub chain assembly complex (LUBAC) and counteracted by OTULIN. Besides cell survival, M1 Ub also controls immune responses, protein trafficking and autophagy, by modifying autophagic cargo. We previously identified novel roles of M1 poly-Ub in controlling the autophagic flux and degradation of cellular cargo, but how OTULIN affects ABC-DLBCL remains unclear. Here, we aim to unravel how OTULIN regulates autophagy induction, degradation of damaged lysosomes and autophagic cell death in ABC-DLBCL. Depletion of OTULIN leads to accumulation of M1 ubiquitin levels in ABC-DLBCL, which was accompanied by increased basal LC3 lipidation. Loss of OTULIN further determines the sensitivity towards cell death induction upon LMP induction. Ongoing molecular analysis of OTULIN will provide new insights into how OTULIN regulates lysosome homeostasis, inflammation and cell survival in ABC-DLBCL.

0072 SNHG29 is a novel megakaryoblastic leukemia specific lncRNA dependency

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Acute Megakaryoblastic Leukemia (AMKL) represents a poor prognostic subtype of acute myeloid leukemia, necessitating novel treatment strategies. Through comprehensive expression profiling of AML patient blasts and healthy bone marrow, we identified a distinct long non-coding RNA (lncRNA) signature overexpressed in AMKL. CRISPRi screening targeting this signature revealed SNHG29 as specifically essential for the proliferation of M-07e, an AMKL cell line. Orthogonal validation via shRNA and CasRx knockdown replicated this finding, suggesting a transcript-dependent mechanism. The effect extended to diminished colony-forming ability in AMKL patient-derived xenografts in vitro and, strikingly, complete depletion in competition assays in vivo. RNA pulldown assays demonstrated SNHG29's interaction with RNA master regulator XRN2. RNA-Sequencing revealed XRN2 RNA targets are upregulated following SNHG29 knockdown. We identified SNHG29 as a key dependency in megakaryoblastic leukemia. SNHG29's interaction with XRN2 highlights its pivotal role in RNA dysregulation in AMKL. Our findings suggest targeting SNHG29 could offer significant therapeutic benefits for this leukemia subtype.

0073 Spatial and temporal tumor heterogeneity representation in liquid biopsies of pediatric precision oncology patients

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Biopsies of high-risk pediatric tumors are challenging and often provide limited molecular insights due to spatial and temporal heterogeneity. Liquid biopsies, particularly cell-free DNA (cfDNA) analysis, offer a non-invasive alternative to detect tumor subclones and refine diagnostics. This study evaluates cfDNA as a liquid biopsy analyte to improve non-invasive tumor characterization and detect mutations. As part of the INFORM program, cfDNA from 130 patients was analyzed using optimized extraction, whole-genome, methylome, whole-exome, and targeted sequencing. cfDNA data were compared with tissue analyses to improve the characterization of tumor heterogeneity. Low-coverage

whole-genome sequencing (lcWGS) showed high sensitivity for circulating tumor DNA (ctDNA) detection. Combining fragment length analysis with copy-number-variation (CNV) profiling improved plasma-based tumor detection, enabling non-invasive identification in 93 % of patients. In a Wilms tumor patient, liquid biopsy identified subclones that were missed by tissue biopsy. cfDNA analysis provides insights into tumor heterogeneity, detects previously hidden subclones, and may reveal new therapeutic targets.

0074 Minimal Invasive Detection of Circulating Biomarkers in Pediatric Cancer Patients Undergoing Chimeric Antigen Receptor

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Pediatric tumors pose therapeutic challenges due to genetic heterogeneity and limited precision oncology targets. Chimeric Antigen Receptor T-Cell (CART) therapy has recently emerged as an option when other treatments fail. Traditional tumor monitoring relies on invasive biopsies, limiting therapy response assessment frequency, while liquid biopsies (LB) offer a minimally invasive alternative for dynamic tumor evaluation. This study tracks CART therapy response in pediatric cancer through serial LBs to analyze tumor cell turnover and molecular changes. Cell-free DNA (cfDNA) was isolated from cerebrospinal fluid (CSF) before, during, and after therapy application and analyzed using low-coverage whole-genome sequencing, methylation sequencing, and proteome profiling. Tumor as well as cfDNA-specific alterations, CNV patterns, and dynamic methylation changes enabled time-resolved tumor evolution analysis. Proteome data detected CAR target expression. Long-term minimal residual disease detection after successful therapy demonstrated that LBs complement standard-of-care MRI diagnostics. Their integration into future CART trials is crucial to assess full prognostic value.

0075 Analysis of differential RNA expression suggests a key role of HDAC1 and let-7 in the pathogenesis of pineoblastoma

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Pineoblastoma (CNS WHO Grade 4) represents a rare, highly aggressive pediatric tumor of the pineal gland. Mutations of components of miRNA processing pathways including DROSHA, DICER1 and DGCR8 as well as non-miRNA related genes like RB1 as well as upregulation of OTX2 and OTX3 are linked to pineoblastoma, but the impact of such alterations on mRNA as well as miRNA expression patterns has not been studied in detail. A concept on the pathogenesis of pineoblastoma has yet not been defined. Using the NanoString method, we compared the mRNA and miRNA expression of 29 pineoblastomas to 24 non-WNT / non-SHH medulloblastomas and 4 non-neoplastic pineal samples. RNA was extracted from formalin-fixed, paraffin embedded tissue from the archives of the DGNN Brain Tumor Reference Center in Bonn (Germany). In pineoblastoma, HDAC1-mRNA was found overexpressed and members of let-7-miRNA family were downregulated compared to medulloblastoma samples. The proliferation of pineoblastoma cells in culture was inhibited after siRNA-mediated HDAC1 knockdown or treatment with HDAC-inhibitors. Our finding may hint to a novel therapeutic target present in pineoblastomas.

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