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Tagungspräsidenten:
Prof. Dr. Martin Stanulla, Medizinische Hochschule Hannover,
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0001 Evaluation of Navitoclax (ABT-263) as a promising Therapy for Pediatric Acute Myeloid Leukemia

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Despite therapeutic advancements for pediatric AML, relapse rate remains high, particularly for leukemia with KMT2A aberration and Down syndrome-associated leukemia (ML-DS). This preclinical study explores Navitoclax (ABT-263) as a potential treatment for these vulnerable patient collectives. Four AML cell lines and leukemic blasts from eight pediatric AML patients underwent escalating ABT-263 exposure, resulting in significant growth reduction (EC50 values: 93 nM). A therapeutic range (EC50: 1.2 µM in CD34 + cells) was identified. Western blot and shRNA experiments unveiled variable BCL-2 family expression profiles, while BH3 profiling illuminated apoptotic interactions. In vivo experiments demonstrated a favorable response and survival advantage in KMT2A aberration after a 21-day treatment, while ML-DS remained unresponsive to ABT-263. ABT-263 emerges as a promising therapy for KMT2A aberration, with BH3 profiling aiding therapeutic response estimation. By highlighting apoptosis dysregulation in childhood AML, this study suggests unexplored therapeutic avenues, contributing to the optimization of treatments for high-risk pediatric AML.

0002 The role of lncRNAs in fusion-oncoprotein containing oncogenic biomolecular condensates in Acute Myeloid Leukemia

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NUP98-fusion oncoproteins, which are found in aggressive forms of pediatric leukemia, form biomolecular condensates in the nucleus of AML cells, and these structures are critical for oncogenic gene expression. We aimed to investigate the contribution of cellular long noncoding RNAs (lncRNAs) to biomolecular condensation of the NUP98::KDM5A fusion oncoprotein. We developed biotinylated isoxazole-mediated condensome RNA-sequencing (biCon-Seq) to identify RNAs present in oncogenic condensates. We calculated a solubility score that is based on the abundance ratios of RNAs in different cellular environments and classified RNAs according to their different solubilities. Expression of NUP98::KDM5A strongly affected RNA solubility, as biCon-Seq after dTAG-induced NUP98::KDM5A degradation revealed a global shift in RNA solubility. We are currently characterizing the roles of several lncRNA candidates which were enriched in biomolecular condensates in a NUP98::KDM5A-dependent fashion. We propose that the NUP98::KDM5A fusion oncoprotein-dependent lncRNA composition of biomolecular condensates is essential to establish and maintain leukemic gene expression.

0003 Molecular differences of paired primary and recurrent atypical teratoid/rhabdoid tumors (AT/RT)

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Atypical teratoid/rhabdoid tumors (AT/RT) are the most common malignant brain tumors during infancy. They are associated with a dismal outcome, and 50 % of the patients suffer a progress or recurrence of the tumor. Molecular characteristics of disease recurrence are currently unclear. We investigated tumor tissue from 26 patients to identify signatures of recurrences in comparison to matched primary tumor samples using histology, DNA methylation analysis, and RNA sequencing. This revealed differing copy number variations (CNVs), showing novel gains on chromosome 1q or losses of chromosome 10 in recurrences. According to bulk RNA sequencing analysis, a number of genes involved in tumor growth, embryonal development, and cell cycle showed significantly altered expression in AT/RT-SHH recurrences. Additional single-cell RNA sequencing resulted in a separation of primary and recurrent tumor cells in specific clusters. Overall, molecular changes that occur in the course of the disease were identified, which may help to define new therapeutic targets for AT/RT recurrences.

0004 Exosome-liposome hybrid system for reprogramming medulloblastoma and glioblastoma immunosuppressive microenvironment

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The immunosuppressive character of Medulloblastoma (MB) and Glioblastoma (GBM) tumor microenvironment (TME) has hindered the development of effective targeted immunotherapy. It is now clear that a successful immunothe-

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rapy for brain tumors is only feasible through understanding the communication between TME components. In this study, we aim to understand the EV-mediated interaction between TME cells to ultimately develop a non-toxic combinatorial nanotechnology-assisted immunotherapy to reverse immunosuppression in MB and GBM TMEs. We isolated small EVs (Exosomes) from ReN cells by size exclusion chromatography, and characterized them by western blot, NTA, and TEM. Functionally we demonstrated that EVs released from MB tumors promote the vascularity of brain endothelial cells and inhibit the immune activation properties of microglia. Subsequently, targeted liposomes with pH-sensitive lipid components were prepared and conjugated with nanobodies against tumor stromal cells. Exosomes and liposomes were hybridized by ultrasound and extrusion and characterized by TEM and FRET. Targeting ability, cytotoxicity of the hybrid, effects on tumor growth and survival, and tumor modulation are under investigation.

0005 Deciphering the NPM1c epigenetic network in acute myeloid leukemogenesis

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Nucleophosmin 1 (NPM1) gene encodes for a multifunctional nucleolar protein that shuttles between the nucleus and cytoplasm. NPM1 mutations are the most common genetic lesion in AML, accounting for one-third of cases, resulting in a 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 can bind to chromatin at important self-renewal-associated gene loci, such as HOXA/B and MEIS1, and can directly regulate the oncogenic transcription. Now the critical question is which other epigenetic factors cooperate with NPM1c in driving leukemic transformation. Our study delves into the exploration of potential NPM1c interacting epigenetic factors and dependencies of NPM1c AMLs based on hits derived from BioID and CRISPR screenings. The goal of this study is to expand our understanding of AML epigenetics and thereby identify molecular targets for potential new epigenetic therapies.

0006 CRISPR/Cas9-mediated KMT2A-rearrangements for the development of leukaemia mouse models

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KMT2A-chromosomal rearrangements are associated with the onset of acute leukaemia. Here, we present our data about CRISPR/Cas9-mediated chromosomal translocations. We either created t(4;11) or t(6;11) chromosomal translocations in umbilical cord blood (UCB) hematopoietic stem and progenitor cells which were transplanted into NSG mice. Many parameters regarding sgRNAs, transfection and culture conditions have been optimized in order to allow leukemic stem cell outgrowth. We did not observed any donor dependency as each UCB donor had the potential to generate CRISPR/Cas9-mediated t(4;11) and t(6;11) chromosomal translocations. In vivo experiments demonstrated rapid engraftment, oligoclonal expansion in bone marrow, liver and spleen. We can conclude that our optimized CRISPR/Cas9-system results in valuable mouse models that develop Leukemia within a few weeks and allows rapid research on different MLL-rearranged fusion proteins.

0007 Naturally occurring antibodies as next-generation immunotherapeutics for high-risk neuroblastoma

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Neuroblastoma (NB), the most common extracranial solid tumour in children, presents a major clinical challenge for paediatric oncology. Despite advances in multimodal therapies, high-risk NB still has a poor prognosis and a high relapse rate, necessitating the development of novel therapeutic alternatives. In a recent study involving a cohort of 248 healthy individuals, we made an interesting discovery. Around 5 % of the participants possessed naturally occurring antibodies (nAbs) within their serum that induced potent complement-mediated cytotoxicity in NB cells. We identified the antigen of these nAbs and confirmed their target specificity. Using the 10X barcode enabled antigen mapping (BEAM) technology, we identified antigen-specific B cell clonotypes of nAb + donors, including their corresponding V(D)J sequences. These sequences are now used to produce recombinant antibodies, that will be characterized for their specificity and effectiveness against NB cells. Our findings hold great promise for the development of a new immunotherapeutic option. However, further research is needed to assess the antibody potency in preclinical and clinical settings.

0008 Replication stress associated micronucleation of extrachromosomal DNA

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Extrachromosomal DNA (ecDNA) is a potent vehicle for oncogene amplification in aggressive cancers. However, the mechanisms that can cause their sequestration in micronuclei during mitosis and its functional consequences for oncogene regulation are currently incompletely understood. Here we show that ecDNA micronucleation occurs more frequently than would be expected by random mitotic mis-segregation. Intriguingly, high ecDNA content, which was associated with increased levels of DNA damage and replication stress on ecDNA, was accompanied by higher levels of ecDNA micronucleation. In contrast to prior assumptions, replication stress resulted in increased ecDNA copy numbers, suggesting that micronucleation does not directly induce ecDNA loss. Importantly, chromatin in ecDNA-containing micronuclei was repressed, raising the possibility that micronucleation may affect oncogene regulation. This demonstrates that ecDNA are prone to micronucleation and that this process can be pharmacologically promoted. Targeting this process has the potential to transform therapeutic strategies for oncogene silencing in cancers.

0009 Deciphering the role of KANSL1 mutations in the development of Myeloid Leukemia in children with Down Syndrome (ML-DS)

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Transient abnormal myelopoiesis (TAM) is a form of clonal hematopoiesis seen in children with trisomy 21. TAM is caused by mutations in the transcription factor GATA1, leading to the expression of a shortened isoform (GATA1s). TAM clonally progresses to myeloid leukemia in Down syndrome (ML-DS) upon acquisition of secondary mutations. Leveraging a virus-free CRISPR screening

platform, GATA1s and additional mutations were introduced into primary human fetal liver hematopoietic stem and progenitor cells (hFL-HSPCs). In vitro testing 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. We revealed domains of KANSL1 essential for normal hematopoiesis and leukemic growth. Moreover, we aim to describe KANSL1 interactome to gain insight into its contribution to the epigenome and gene expression. This knowledge will help us further define the impact of mutated KANSL1 on downstream pathways. Future research will explore potential new therapeutic vulnerabilities in ML-DS patients carrying mutated KANSL1.

0010 Exploiting MYB-dependent vulnerabilities for novel therapeutics in acute myeloid leukaemia

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Despite extensive research, 30 % of paediatric AML cases still experience relapses, prognosis and treatment options for these AMLs are still unfavourable and limited. AML often exhibits dependencies on driver/oncogene aberrations and on downstream oncogenic mediators for survival and progression, for example, MYB. However, targeting MYB remains elusive due to its "undruggable" nature as a transcription factor. Our project aims to identify MYB's role and its co-factors particularly those with potential as novel therapeutic targets in AML. Using CRISPR-Cas9 dropout screen on MYB-overexpressing cells, we revealed alterations in metabolic pathways, especially those involved in glucose utilisation, as crucial for MYB-driven AML. A higher level of extracellular lactate was detected in MYB-overexpressing cells and vice versa in MYB-silenced cells. Interestingly, the first rate-limiting enzyme in glycolysis, HK2, and glucose transporter, GLUT1 emerged as essential genes in MYB-overexpressing cells. Our study suggests targeting glucose metabolism, especially in MYB-overexpressing cases, holds promise for AML therapy.

0011 Investigating the role of OTULIN and the regulation of linear ubiquitination in the pathogenesis of ABC-DLBCL

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Aberrant NF-κB activation has been identified as a hallmark of Activated B-Cell-like Diffuse Large B-Cell Lymphoma (ABC-DLBCL) and underlies proliferation, survival and inflammation. The assembly of linear ubiquitin chains is catalysed by the linear polyubiquitin chain assembly complex (LUBAC) which is counteracted by the deubiquitinating enzyme OTULIN. Besides linear ubiquitin, B cell receptor (BCR) signaling and the CARD11-BCL10-MALT1 (CBM) complex emerge as pivotal regulators that finetune NF-κB activation and drive lymphoma. Here, we aim to unravel the functions of OTULIN in the pathogenesis of ABC-DLBCL. OTULIN deficiency in ABC-DLBCL cells leads to accumulation of linear ubiquitination and dysregulation of NF-κB activation. Loss of OTULIN further determines sensitivity towards cell death induction by cytokines, Smac mimetics and MALT1 inhibition. Ongoing molecular analysis of OTULIN targets will provide new insights in how OTULIN regulates NF-κB addiction in ABC-DLBCL. Understanding the interplay between OTULIN, BCR pathway proteins and cell death might be crucial for developing effective therapeutic strategies targeting ABC-DLBCL.

0012 The activation of TP53 pathway is a therapeutic vulnerability in NUP98::KDM5A + Pediatric Non-Down Syndrome AMKL

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The NUP98::KDM5A (NK5A) translocation occurs in ~15 % of pediatric non-Down syndrome AMKL (non-DS-AMKL), is associated with young age, and correlates with poor prognosis. We explored the fetal cellular origin of NK5A-driven leukemia for novel treatment options and discovered Trip13 dependence. Functionally, loss of Trip13 caused dramatic upregulation of TP53 protein and cellular response. Mechanistically, TRIP13 binds WIP1/PPM1D, a regulator of TP53 abundance and putative mediator TRIP13-dependent TP53 control. Functionally, loss of Ppm1d phenocopied loss of Trip13 in NK5A leukemic cells, and Trip13 loss was rescued by ablation of Trp53 or Bbc3/Puma. Based on these findings we reasoned that inhibition of MDM2 (Idasanutlin) and BCL2/BCL-XL (Navitoclax), in conjunction with the broadly applicable chemotherapy sensitizer Azacytidine, may hold a novel treatment approach for NK5A-AMKL. Indeed, highly synergistic induction of NK5A-leukemic cell death was observed with Idasanutlin/Navitoclax/Acacytidine. In summary, the fetal origin of NK5A non-DS-AMKL revealed the TRIP13-WIP1-TP53-axis and Idasanutlin/Navitoclax/Acacytidine as a molecularly-guided therapy option.

0013 Polyphosphates are regulators of metabolic homeostasis upon amino acid starvation in drug resistant leukemia cells

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In leukemia cells, tolerance of nutrient deprivation can be driven by GSK3α, which undergoes supramolecular assembly with the ubiquitin-proteasome system to form so-called GSK3α bodies. This process drives protein degradation to generate an alternative source of amino acids, an adaptive response co-opted by cancer cells for asparaginase resistance. In this context, we could recently identify the essentiality of the protein PRUNE, which mediates GSK3α body formation in dependence on its exopolyphosphatase activity. These findings thus raise the question whether polyphosphate levels are regulators of supramolecular assembly in leukemia cells. Intriguingly, we could demonstrate that the phase-separating N-terminal domain of GSK3α displays a positively charged surface, which interacts with negatively charged polyphosphates through electrostatic non-covalent forces. This interplay blocks formation of GSK3α bodies, and thus the ability of leukemia cells to survive an amino acid shortage. Notably, levels of polyphosphates significantly correlated with response and resistance to asparaginase, providing a rationale to further assess the value of polyphosphates as a potential biomarker.

0014 Results of the RIST-rNB-2011 trial for relapsed or refractory neuroblastoma

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Relapsed or refractory neuroblastoma (r/rNB) are associated with a dismal outcome.

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RIST-rNB-2011, an open label randomised phase II trial (NCT01467986) compared RIST (rapamycin, dasatinib, irinotecan, temozolomide) with I/T alone. 129 patients with relapsed high-risk and refractory NB were randomly assigned to RIST or I/T, stratified by MYCN. Primary endpoint was PFS. 99 (80 %) were relapsed and 24 (19 %) refractory NB patients. At a median follow-up of 72 months the median PFS was 11 (95 %-CI 7-17) months in RIST and 5 (2-8) months in CA (HR: 0 · 62 [95 %-CI: 0 · 42, 0 · 92], p = 0 · 019). Median PFS with RIST in MYCN-A patients was 6 (95 %-CI 4-24) months versus 2 (2-5) months in the CA (HR: 0.45 (0 · 24, 0 · 84), p = 0 · 012). PFS in the MYCN-A subgroup for RIST versus CA was 33 % versus 8 % at 2 years. Median OS with RIST in MYCN-A patients was 11 (95 %-CI 7-26) months versus 6 · 5 (95 %-CI 3-15) months in the CA (HR: 0 · 51 (95 %-CI: 0 · 27, 0 · 96), p = 0 · 037). No difference in outcome was observed in the MYCN non-amplified (MYCN-NA) patients. Conclusion: RIST demonstrated a significant and sustained anti-tumour activity, selectively in the highest-risk group of MYCN-A patients with r/rNB.

0015 CD127-targeted immunotherapy outperforms Imatinib in preclinical models of ABL-class fusion positive BCP-ALL

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Current therapy strategies for B cell precursor acute lymphoblastic leukemia (BCP-ALL) are mainly based on polychemotherapy. ABL-class fusion-positive (ABL-fusion +) BCP-ALL is associated with poor outcome and additionally treated with tyrosine kinase inhibitors such as Imatinib (Ima). Ima, in combination with chemotherapy, can cause excessive toxicity. Immunotherapy (IT) targeting IL-7Rα (CD127) has shown promising results in preclinical models of BCP-ALL. Therefore, we tested CD127-targeted IT in ABL-fusion + BCP-ALL models. We detected CD127-positivity in the majority of ABL-fusion + cases in prospective flow cytometry measurements. Moreover, we found that a CD127-antibody induced antibody-dependent cellular phagocytosis in ABL-fusion + cell lines and patient-derived xenograft (PDX) cells in vitro, particularly when combined with Ima. In a phase2-like preclinical trial, we detected a reduction of ALL-burden in 8/8 PDX-models, including a CD19-knockout PDX. A significant survival prolongation was also observed. Of note, CD127-IT thereby significantly outperformed Ima in vivo. Altogether CD127-IT can be an effective option in ABL-fusion + BCP-ALL warranting clinical investigation.

0016 Reverse engineering BCP-ALL signaling with large knock-out screens

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Modern screening protocols using CRISPR allow the generation of high content knock-out response datasets. CRISPR knock-out (KO) screens [Hart2017] measure the dependency of a cell line to specific gene knock-out. Repeated over multiple cell lines with recurrent alterations and with drugs allows the extraction of biomarkers driving genetic dependencies that can be combined to improve therapeutic decisions.
CROPseq [Datlinger2017] couples CRISPR knock-out and single cell RNA sequencing to measure the transcriptome of a single gene knock-out in a pooled setting, allowing the simultaneous measurement of the transcriptomic response to dozens of KO in parallel at single cell resolution. Using public and in-house large knock-out screens combined with our CROPseq dataset in three B-ALL cell lines, we reconstructed the signalling and dependency landscape of acute lymphoblastic leukemia. We reconstructed the key regulatory modules in those cell lines, and expanded the regulatory networks to the main downstream targets. We show that the B-cell specific regulation of the PI3K pathway is conserved in B-cell ALL and presents a multi-actionable therapeutic opportunity.

0017 γ/δ-T cells for the immunotherapy of pediatric acute lymphoblastic leukemia with blinatumomab

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Immunotherapy with the bispecific T-cell engager blinatumomab has greatly improved survival of children with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (BCP-ALL) by providing a bridge to hematopoietic stem cell transplantation (HSCT). Relapses shortly after HSCT still remain extremely challenging and these patients have a poor prognosis. In patients after HSCT, especially when receiving T cell depleted grafts, immune reconstitution of α/β-T cells is often very slow. The mechanism of action of blinatumomab however requires the presence of sufficient CD3-positive effector T cells. We show the ability of ex vivo expanded γ/δ-T cells by healthy donors to lead to blinatumomab-induced cytotoxicity against BCP-ALL cell lines in vitro with low effector to target ratios. γ/δ-T cells recognize their target antigens independently of the major histocompatibility complex, their activation does therefore not result in graft versus host disease and would allow the production of "third party" cellular products. Blinatumomab in combination with γ/δ-T cells might provide a treatment option with a low toxicity profile for early BCP-ALL relapses post HSCT.

0018 The nanoparticle-mediated delivery of therapeutic siRNA's targeting fusion genes in leukemia

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Acute myeloid (AML) and acute lymphoblastic (ALL) leukemias can be characterized by chromosomal rearrangements, resulting in fusion-oncoproteins that can block hematopoietic differentiation and induces aberrant gene expression. Therapeutic siRNAs can effectively target these gene fusions, however effective delivery and siRNA degradation limit their application. Lipid nanoparticles (LNPs) have been reported to safely and effectively deliver siRNAs to target cells. Here, we investigated the delivery of siRNA's targeting the fusion junctions MLL-AF4 in ALL, and MLL-AF6 in AML cells by utilizing LNP formulations with differences in lipid composition and molar ratios. Furthermore, we assessed the uptake efficacy of LNPs and target gene knockdown. We also investigated the efficacy of LNPs with and without a targeting moiety for the recognition of the VLA-4 receptor on hematopoietic cells. We observed differences in LNP uptake and siRNA-mediated knockdown efficiency between ALL and AML, where AML cells were more responsive to knockdown following LNP treatment. These differences will allow us to optimize LNP formulations to deliver target siRNAs for the treatment of distinct leukemias.

0019 STAT3β represses interferon signaling in acute myeloid leukemia contributing to favorable disease outcomes

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Dysregulated STAT3 signaling is associated with adverse outcomes in many cancers. STAT3 exists in two alternatively spliced isoforms: the full-length isoform STAT3α and the truncated variant STAT3β. Previously, we identified a link between low STAT3β expression and adverse outcome in acute myeloid leukemia (AML). We here describe the mechanism behind its protective role and provide potential therapeutic strategies.

We established a mouse model of STAT3β-deficient, MLL-AF9-driven AML. Absence of STAT3β in leukemia cells leads to significantly reduced survival in mice confirming STAT3β's tumor suppressive role. RNA sequencing of leukemia cells from diseased mice revealed enhanced STAT1 and interferon (IFN) signaling in the absence of STAT3β. STAT3β-deficient leukemia cells were more susceptible to inhibition of IFN signaling by the JAK1/2 inhibitor Ruxolitinib. Analysis of patient samples and publicly available data revealed a link between low STAT3β expression and elevated IFN signaling and adverse outcome. Our study highlights STAT3β's tumor suppressive role in AML by damping IFN signaling. Thus, patients with low STAT3β levels may benefit from therapies targeting IFN signaling.

0020 Adaptive NK cells in combination with tyrosine-kinase inhibition for the treatment of Ph- like ALL

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NK cells are potential immunotherapy tools for childhood leukemia, in particular epigenetically reprogrammed memory-like NK cells. Studies on DNA methylation patterns in activated NK cells might identify activation biomarkers and strategies to enhance cytotoxicity. The DNA methylation profile of NK cells examined in canonical and expanded NK cells, generated by a 7-day culture with K562 cells expressing membrane bound IL21/4-1BB. Memory-like features achieved by 16-hour stimulation as previously published. DNA and RNA were analysed using Illumina methylation bead array and Illumina NextSeq2000. Bioinformatics analyses revealed distinct methylation patterns in preactivated and expanded NK cells, and enrichment of genes encoding ribosome and mitochondrial function, immune system process, cell adhesion and motility. A crosswise comparison of differential gene regulation based on canonical NK cells revealed a substantial overlap of genes encoding PD-1 and IFN signaling, MHC class II and TNF-NFKB signaling. Ruxolitinib disrupted memory formation and cytotoxicity in JAK class Ph-like ALL, while a humanized CD19 mAb rescues TKI-induced NK cell function deficit via FcγRIIIa mediated ADCC.

0021 Discovery of Oncogenic Mechanisms regulated by EZH2-regulated RBPs in AML

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Enhancer of zeste homolog2 (EZH2) is the core catalytic subunit of polycomb repressive complex (PCR) 2, which regulates development and differentiation of hematopoietic stem cells in concert with PRC1. Loss of PRC2 activity is a frequent event in the progression of adult myelodysplatic syndromes and Down syndrome associated AML. Amongst the PRC2-regulated genes are a plethora of RNA-binding proteins (RBPs), as highlighted by our previous studies of Ezh2-mutated murine AML models. While RBPs are major drivers of post-transcriptional gene regulation and major implications in malignant transformation have been recognized, it is unknown how PRC2-regulated RBPs may shape the post-transcriptional landscape in hematopoietic malignancies. We thus hypothesized that RBPs represent a feed-forward mechanism, which mediates AML transformation upon loss of epigenetic regulators. By utilizing a high content lentiviral CRISPR library targeting 1542 RBPs, and combined screening of cell lines and primary cells in a PRC2 -dependent manner, we aim to characterize the PRC2-RBP transformation axes in AML, and identify its treatment relevant weaknesses for future targeted therapy.

0022 Inhibition of Cyclin-dependent Kinase 9 Leads to Rapid and Effective Apoptosis Induction in Acute Lymphoblastic Leukemia

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Using drug response profiling in B-cell precursor (BCP) and T-cell acute lymphoblastic leukemia (ALL) cell lines and primary, patient-derived xenograft (PDX) samples, we observed potent activity of the CDK1,2,5 and 9 inhibitor dinaciclib in leukemias of both lineages. Surprisingly, we did not find changes in cell cycle and cell proliferation but found rapid apoptosis induction characterized by Annexin V/PI double-positive cells, dose-dependent activation of caspase-3/7 and PARP, reduction of phospho-RNA polymerase II (p-RNAP-II) and decreased levels of the pro-survival protein MCL-1, pointing to cell death induction via the inhibition of CDK9. Consistent with these observations, the specific CDK9-inhibitor ADZ4573 resulted in rapid apoptosis induction with caspase-3/7 activation and PARP-cleavage, along with decreased p-RNAP-II and MCL-1 protein expression. Importantly, we found that combining CDK9 inhibition with inhibitors of BCL-2 and/or BCL-XL results in a largely synergistic cell death induction. Taken together, we identified strong anti-leukemia activity by CDK9 inhibition with rapid apoptosis induction in BCP- and T-ALL, warranting further pre-clinical evaluation.

0023 Glyco-binding domain chimeric antigen receptors as a new option for cancer immunotherapy

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A conventionel CAR consists of an antibody fragment designed to specifically recognize target structures on cancer cells and intracellular signaling domains. For many cancer entities, a suitable antigen is missing or no monoclonal antibody is available for the construction of a CAR. We show that, lectin-glycan interactions are suitable for use in immunotherapy. Cancer cells are often characterized by an altered glycosylation pattern, which can be recognized by lectins. Replacing the antibody fragment with the glyco-binding domain of a human lectins in a CAR construct leads to targeted cancer cell killing. Using the glycan-binding properties of the CD301 receptor, we have developed a novel type of CAR (LEC-CAR) that enables cytotoxic effector cells to recognize Tn and sialyl-Tn antigens and thus eliminate cancer cells. In addition, we investigated the glycosylation profile of acute myeloid leukemia (AML) cell lines and patient material. Based on this, we developed more CAR constructs to target this can-

cer entity and could show a specific killing in vitro. Our novel LEC-CAR concept offers several advantages: broader applicability, short generation time, and low immunogenicity.

0024 A structurally improved Decitabine analogue induces increased anti-leukemia activity in vivo

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Hypomethylating agents like DNMT-inhibiting antimetabolites are an important approach to treat patients with acute myeloid leukemia (AML), though, their efficacy is limited by drug induced adverse effects. We aimed to design and test a more efficient and less toxic analogue of Decitabine. In tests in AML cell lines, the new Decitabine analogue cAzadC revealed higher stability, a higher amount of double strand breaks, increased inhibition of DNMT1 and suppressed cell proliferation. To compare both efficacy and toxicity between Decitabine and cAzadC, we performed *in vivo* trials using the patient derived xenograft (PDX) mouse model. Mice tolerated 10-fold higher doses of cAzadC without signs of toxicity, indicating that it induces decreased adverse effects. The overall anti-leukemia efficacy was higher and notably, some mice were long-term cured. Our results show that cAzadC combines improved anti-leukemia activity with reduced toxicity *in vivo*. Improving the structure of DNMT-inhibiting antimetabolites leads to a favorable balance between efficacy and toxicity, which might benefit AML patients for more efficient treatment in the future.

0025 Effects of the allosteric tyrosine kinase inhibitor asciminib on bone metabolism

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Chronic myeloid leukemia (CML) treatment using tyrosine kinase inhibitors (TKIs) poses impaired longitudinal growth in pediatric patients. Alternative therapeutic concepts such as a more selective inhibition of ABL1 kinase by the allosteric inhibitor asciminib could be particularly beneficial in children. We therefore compared in vitro the impact on bone metabolism of asciminib to the current first- and second-line TKIs imatinib and dasatinib.
Asciminib had a lower impact on osteoclast viability, differentiation, and function and did not affect the mineralization capacity of osteoblasts in contrast to the other TKIs.
While imatinib and dasatinib significantly inhibit osteoclast differentiation and function, no significant effects were observed for asciminib. Our findings suggest asciminib as an ABL1 kinase inhibitor with fewer side effects on bone metabolism, potentially improving longitudinal growth.

0026 Deciphering kinase dependent and independent functions of CDK8 in lymphoid and myeloid leukemia

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Cyclin-dependent kinase 8 (CDK8) is under consideration as a target for cancer therapy. Kinase independent functions of CDK8 are critical for B cell Acute Lymphoblastic Leukemia (B-ALL). In contrast, it has been shown that CDK8 kinase inhibition leads to an anti-proliferative effect in acute myeloid leukemia (AML) cells. This underlines the divergent functions of CDK8 in leukemia. To address kinase dependent and independent functions of CDK8 we generated a mouse model expressing either wild-type CDK8 (wt), kinase inactivated CDK8 (CDK8D173A) or lacks CDK8. No major influence of CDK8 kinase inactivity on normal hematopoiesis has been observed. To compare the role of CDK8 in lymphoid and myeloid leukemia, we established bone marrow derived cell lines expressing either BCR-ABLp185 or MLL-AF9-NRAS. An increased apoptosis, mTOR signaling and lysosomal activity was determined in CDK8D173A and CDK8 knockout p185 + cells. Lysosomes regulate several cellular processes, e.g. migration, plasma membrane repair and vesicle regulation. This project deepens our knowledge about the role of CDK8 in leukemic transformation and progression and its potential as a therapeutic target.

0027 A Novel Fc-Optimized Antibody Drug Conjugate Targeting CD7 is Active in Preclinical Models of T-ALL

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Despite progress in the treatment of T-ALL, immunotherapeutic options remain limited. The CD7 antigen represents an ideal target structure for the development of antibody drug conjugates due to its high internalization capacity. Here, a CD7 antibody was optimized for its ability to trigger ADCC/ADCP and conjugated to the microtubule-disrupting agent MMAE via an enzymatic-cleavable linker (CD7-DE-vcMMAE).
CD7-DE-vcMMAE eliminated T-ALL cells through ADCC by NK cells or ADCP by macrophages. In addition, CD7-DE-vcMMAE showed strong direct cytotoxic effects by delivery of the cytotoxic compound to T-ALL cells (IC 50 < 1 nM). In a subcutaneous T-ALL xenograft NSG model using CEM cells, CD7-DE-vcMMAE reduced tumor growth significantly. Furthermore, CD7-DE-vcMMAE prolonged the survival of mice in a preclinical phase II-like xenograft study employing eight samples from pediatric and adult patients. Importantly, animals treated with CD7-DE-vcMMAE that survived the experimental period were free of minimal residual disease. These results exhibit CD7-DE-vcMMAE as a promising therapeutic strategy for T-ALL.

0028 Inhibition of small EVs secretion and its implication on cytoplasmic DNA sensing and cancer immunogenicity in AML

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Cancer cells secrete small extracellular DNA (sEVs) with higher DNA (EV-DNA) content than their normal counterparts. However, the question remains: How is EV-DNA selected for secretion in sEVs, and under which circumstances? Here, we studied the implication of inhibiting sEVs secretion on cytoplasmic DNA (cyDNA) accumulation, cyDNA sensing, and cancer immunogenicity in acute myeloid leukemia (AML). To inhibit sEVs secretion, we generated Rab27a-knockout blasts or treated the cells with the farnesyltransferase (FT) and sphingomyelinase (SP) inhibitors Manomycin A and GW4869 prior to the treatment with DNA damage-inducing agents Cytarabine (Ara-c) or hydroxyurea (HU). We observed a substantial accumulation of cyDNA with concomitant activation of DNA sensor cGAS and a significant increase in IRF3-S386 phosphorylation in AML blasts. Analysis of DNA damage markers revealed a specific increase in YH2A.X nuclear foci and a high expression of the exonuclease TREX1 and DNase I. The NK cells receptor (NKG2D-L) ligand ULBP1 expression was significant-

ly upregulated upon cyDNA accumulation in AML cells. This has significantly increased NK cell toxicity against AML blasts in 2D and 3D cultures.

0029 Comparison of CD123- and CD33-CAR-NK cell preparations in a xenograft mouse model for treatment of AML

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CAR-engineered Natural Killer (NK) cells provide a promising option for donor independent treatment of hematological malignancies, like acute myeloid leukemia (AML). Recently, we reported on the anti-leukemic efficacy of CD33-CAR-NK cells. However, other established AML targets, such as CD123 are also showing promising results. In this study, we compared a CD123-CAR-NK cell product generated by an automated process with CD123-CAR- and CD33-CAR-NK cells prepared by conventional manufacturing. Additionally, the effect of different percentages of CAR expressing cells in the final cell product was studied in vivo and in vitro. In vivo imaging revealed an overall reduced leukemic burden in the treatment groups, compared to the non-transduced NK cell group. Those findings were mirrored by flow cytometry data of in vitro cytotoxicity assessment and were independent of the manufacturing method or the percentage of CAR expressing cells. Notably, CD33 targeting by CAR-NK cells showed less pronounced effects, compared to CD123 targeting. Our results show that the automatized manufacturing of CD123 CAR-NK cells, provides a promising cell therapeutic concept for treatment of AML.

0030 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. Ongoing research will further elucidate the contribution of fetal genes to leukemia pathogenesis. This study underscores the potential of targeting fetal-origin vulnerabilities in infant AML, paving the way for innovative treatment strategies.

0031 Integrative single-cell multi-omics of CD19-CAR_{pos} and CAR_{neg} T cells suggest drivers of immunotherapy response in B-cell neoplasias

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The impact of phenotypic, clonal, and functional heterogeneity on clinical outcomes in CAR-T cell therapy remains understudied. Here, we integrated clonal kinetics and transcriptomic heterogeneity by single-cell omics. We longitudinally examined CARneg and CARpos T-cells in the manufactured IP and in-vivo at the peak of CAR-T cell expansion in 5 B-ALL patients treated with CD19CAR-T. Significant differences were observed in cellular dynamics in response to therapy. CARpos T-cells in the IP displayed a significantly higher CD4:CD8 ratio compared to CARneg T-cells, and this composition of CD4:CD8 CARpos T-cells impacted therapy outcome, as validated in a larger cohort of 24 varni-cel B-ALL patients. Conversely, an inverse trend was consistently noted at the expansion peak, with clonally expanding CD8 + effector memory and cytotoxic T-cells. Cytotoxic CARpos γδT cells emerged at the expansion peak, and their in vivo expansion positively correlated with treatment efficacy, validated in n = 18 cohort of B-ALL patients and n = 58 B-CLL. Our data provide insights into the complexity and diversity of T-cell responses following CAR-T cell therapy and suggest drivers of immunotherapy response.

0032 Targeted modulation of HDAC7 improves the outcome of hematological malignancies

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HDAC7 plays a crucial role in B cell development maintaining cell identity throughout B lymphocyte differentiation. Therefore, its deregulation is linked to hematological malignancies. Infants diagnosed of pro-B acute lymphoblastic leukemia (pro-B-ALL) with t(4;11) chromosomal rearrangement represent a subgroup of patients with poor response to standard therapies and an adverse outcome. Since high levels of HDAC7 entail a significant improvement of their survival, we aimed to establish a precision therapy to restore HDAC7 expression. The administration of this therapy to has demonstrated to reduce leukemogenesis of pro-B-ALL cells in vivo, when applied to patient-derived xenografts. These findings can be translated to other hematological diseases, such as Diffuse Large B Cell Lymphoma (DLBCL), the most common and aggressive type of non-Hodgkin lymphoma. R-CHOP, a combination of chemotherapy and immunotherapy is the standard treatment, but patients with low expression of CD20 are resistant. Remarkably, the induction of HDAC7 in DLBCL cell lines promotes CD20 expression and improves response to R-CHOP treatment, both in vitro and in vivo.

0033 Enhancing Therapy for TP53-Deficient Rhabdomyosarcomas

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Background: Rhabdomyosarcomas (RMS) are prevalent soft tissue sarcomas in children and adolescents, displaying diverse clinical and biological features. Detection of TP53 variants in RMS tissue correlates with dismal prognosis.
Methods: RMS were induced by sorting muscle satellite cells from TP53 TAM-ER/TAM-ER, which were transduced with KRAS (G12v) and injected into NOD. SCID mice. Cell lines were established and exposed to tamoxifen and/or trametinib to activate TP53 signaling and/ or block MAPK signaling. RNA sequencing and high-throughput drug screening using 307 FDA-approved drugs were performed to identify drugs with a preferential effect on TP53-deficient RMS.
Results: RNA sequencing identified protein homeostasis, as a key pathway in TP53-deficient RMS. Activation of TP53 did not change anti-sarcoma efficacy

of 307 candidate drugs. Proteasome inhibitors such as carfilzomib emerged as potent anti-RMS drugs with strong pro-apoptotic effects on mouse and human sarcoma cells irrespective of MAPK signaling. Carfilzomib markedly reduced levels of the proapoptotic effectors BCL-2, BCL-XL and MCL-1 in sarcoma cells. Fibroblasts were mostly resistant to the effects of carfilzomib.

0034 IGF2BP3 as an interesting target gene in t(4;11) leukemia

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The IGF2BP3 gene has been identified in our studies as a central and direct target gene of the hMLL::hAF4 fusion protein. Here, we investigated the biological differences between the human hMLL::hAF4 fusion protein and the murinized version of it, namely the hMLL::mAf4 fusion protein. The latter has been been used by Lin et al., in 2015 to establish a mouse model for human leukemia. However, slight differences exist between the human and mouse AF4 C-terminal protein sequences, which we were aiming to address in functional studies. Both fusion genes were stably transfected via our Sleeping Beauty technology in a reporter cell line (± AF4::MLL), and subsequent experiments were performed. According to our data, IGF2BP3 is a direct target gene of the hMLL::hAF4 fusion protein. Expression of IGF2BP3 is increased, which in turn leads indirectly to the activation of the AF4-MLL fusion protein. Moreover, it seems like there is a direct protein interaction between P-TEFb and the fusion protein in hMLL::hAF4 + AF4::MLL cells, which is missing when compared to the hMLL::mAF4 + AF4::MLL cells. Our results on this issue and the role of IGF2BP3 will be presented and discussed.

0036 A new recombinant oncolytic measles virus for therapy of ALL and assessment of its toxicity

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The CD19xCD3 bispecific T-cell engager (BiTE) blinatumomab is effective against BCP-ALL by engaging CD3 + T cells with CD19 + leukemic blasts. However, its systemic administration can elicit severe side effects and despite additional HSCT long-term overall survival in relapsed/refractory ALL is poor. This new oncolytic measles virus (MV) expressing the anti-CD19 BiTE (MV-Blina) decreases BCP-ALL cell numbers concomitant with increased expression of blinatumomab (secBlina) in infected cells. Purified secBlina from supernatants of infected cells specifically binds to target cells, activates T cells and effectively kills REH BCP-ALL cells in the presence of PBMCs. The elucidation of therapeutic effectiveness of MV-Blina in mice bearing patient-derived xenografts is under investigation. In addition, we addressed the toxicity of MV-Blina in human neuronal cells in vitro. Here, MV-Blina showed a pronounced oncolytic effect on the neuroblastoma cell line SY5Y whereas healthy iPSC-derived human neurons were less affected. The assessment of toxicity in a recombinant susceptible mouse model is ongoing. Altogether, MV-Blina may be a new therapeutic approach in ALL.

0037 Functional characterization of T389 phosphorylation of RNA-binding protein La in neuroblastoma cells

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The overexpression of RNA-binding protein La in various cancer entities including neuroblastoma correlates with poor patients survival. The La protein promotes proliferation, plasticity, chemoresistance, and tumor growth of cancer cells. Interestingly, AKT-dependent phosphorylation of La at threonine 389 (LaT389) regulates its RNA chaperone activity in vitro and facilitates mRNA translation in cell-based assays. Molecular-targeted treatment of neuroblastoma cells with Rapamycin plus Dasatinib, as applied in the RIST therapy, reduces LaT389 phosphorylation. This study aimed to decipher the functional role of LaT389 phosphorylation in cancer cells. Therefore phospho-dead LaT389A and phospho-mimicking LaT389E mutants were generated by CRISPR-based gene editing in neuroblastoma cells. Compared to parental cells, LaT389A expression decreased the viability, proliferation, spheroid formation, and in ovo tumor growth but increased extracellular lactate and acidification. Interestingly, Ribo-Seq analysis revealed reduced translation of mitochondrial proteins and metabolic studies a mitochondrial dysfunction in LaT389A expressing cells. Expression of LaT389E partly reversed the cellular

0038 Replication stress induces extrachromosomal DNA clustering and lagging during anaphase

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Extrachromosomal DNA (ecDNA) is an important driver of oncogene amplification and as such represents an attractive therapeutic target. One potential way to eliminate ecDNA from the cell nucleus may be via incorporation into small nuclear envelopes, a process termed micronucleation. However, molecular mechanisms that could lead to micronucleation of ecDNA remain enigmatic. Here we show that replication stress causes clusters of damaged ecDNA to detach from chromosomes during anaphase, followed by an increase in micronucleation. Unlike mitotic chromosomes, lagging ecDNA clusters showed strong colocalisation with H2AX phosphorylated on serine 139, suggesting either altered repair or increased damage on ecDNA. Overall, this proposes a mechanism for the micronucleation of ecDNA following non-selective DNA damage and illuminates new therapeutic possibilities to increase micronucleation.

0039 Decitabine enhances antibody-dependent effects of Daratumumab immunotherapy in acute lymphoblastic leukemia models

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Current treatment of acute lymphoblastic leukemia (ALL) involves intensive chemotherapy with severe side effects. Options for relapsed/refractory (r/r) cases are limited, particularly in T-ALL, and antibody therapy is a promising alternative. CD38, expressed on both B-cell precursor (BCP)- and T-ALL cells, can be targeted by daratumumab (DARA). The DNA-Methyltransferase1(DNMT1)-inhibitor decitabine (DEC) may enhance CD38 expression on malignant cells, potentially improving DARA efficacy. Therefore, we examined the impact of DEC on the efficacy of DARA in BCP- and T-ALL in vitro. The application of DEC reduced DNMT1-expression and cell viability in BCP- and T-ALL cell lines. CD38 expression under DEC treatment was increased. Moreover, enhanced antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) were observed in BCP- and

T-ALL cell lines after DEC/DARA application. Importantly, DEC/DARA increased ADCP in a variety of patient-derived xenograft (PDX) samples including 4/6 de novo BCP-, 5/5 T-ALL and 5/5 T-PediatricALL PDX samples from r/r disease. Our findings suggest that DEC/DARA is a promising therapy combination in ALL, warranting further investigation in vivo.

0040 Engineering multifunctional GRP78-CAR CIK cells to target relapsed/refractory pediatric AML

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Chimeric antigen receptor (CAR) cell therapy could contribute to a substantial improvement in the survival probability of heavily pretreated patients with relapsed/refractory acute myeloid leukemia (AML), for whom conventional therapies offer a dismal prognosis. We identified the 78 kDa glucose-regulated protein (GRP78) as a highly expressed target on AML cell lines, initial and refractory AML PDXs and primary samples from patients with unfavorable genetic alterations or poor response to treatment. In contrast, GRP78 surface expression was low on healthy hematopoietic and immune cells. Consequently, we designed two second-generation anti-GRP78 CARs containing either Pep42 or SAM-6 scFv as antigen recognition domains. We chose cytokine-induced killer (CIK) cells, a polyfunctional heterogeneous immune effector cell population with T and natural killer (NK) cell properties, as CAR carriers. In a proof-of-concept analysis, their specific elimination of AML cells was significantly enhanced by the expression of a GRP78 CAR. Accordingly, future detailed preclinical efficacy and safety studies are planned to translate novel GRP78-CAR CIK cells into clinical practice.

0041 Functional role of histone variant macroH2A1 (H2afy) in acute myeloid leukemia

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Dysregulation or mutations of histones that exhibit oncogenic features are termed oncohistones. Oncohistones have been described in different cancers. Here, we identify a functional dependency of human AML cells on histone H2A variant macroH2A1 (H2AFY). To validate the functional impact of H2afy in leukemia, we created a conditional knockout mouse for H2afy and established MLL-AF9-driven AML. Deletion of H2afy delayed disease progression and reduced disease penetrance as well as leukemia stem cell (LSCs) numbers. Limiting dilution assays confirmed reduced functional LSCs, consistent with impaired serial re-plating efficiency, reduced colony forming capacity and increased differentiation. This phenotype could be recapitulated when H2afy was degraded using the PROTAG system. In contrast, H2afy-deficient normal HSCs did not show functional defects in serial repopulation assays in vivo. Likewise, colony formation of healthy human CD34 + cells was unaffected by H2AFY deletion. ATAC-sequencing and transcriptome analyses show gain of differentiation related and loss of stemness signatures in H2afy-deficient LSCs. Together, we provide first evidence for a critical role of H2afy in AML.

0042 Characterization of the stromal microenvironment in B-cell Acute Lymphoblastic Leukemia

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The stromal bone marrow microenvironment influences the progression of hematological malignancies, though a complete understanding of its cellular heterogeneity in the context of B-cell acute lymphoblastic leukemia (B-ALL) remains largely unknown. Here, we analyzed patient-derived mesenchymal stromal cells (MSC) and demonstrated their ability to differentiate into osteoblasts, chondrocytes, and adipocytes in vitro. Further, we examined bone marrow cells using single-cell RNA-sequencing and identified two populations of MSCs: one characterized by high fibronectin expression (MSC-1) and the other with high levels of collagen type I (MSC-2). Trajectory analysis indicated that MSC differentiation was polarized from MSC-1 towards MSC-2, accompanied by an increased expression of adipogenic and osteogenic markers. Transcription factor inference suggested that MSC-1 is governed by stem cell regulons, while MSC-2 is regulated by osteogenic ones. Estimation of relative abundance revealed an association between stromal populations and the genomic classification of ALL. Collectively, we showed that the stromal microenvironment in B-ALL is characterized by two distinct MSC cell populations.

0043 YBX1 protein in AML-derived small extracellular vesicles reduces mesenchymal stem cell differentiation to osteoblasts

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Small extracellular vesicles (sEVs) released by AML cells have been reported to influence the trilineage differentiation of bone marrow-derived mesenchymal stem cells (BM-MSCs). However, it remains elusive which cargo from AML-sEVs is responsible for this effect. In this study, sEVs were isolated using size-exclusion chromatography and ultrafiltration. Our results demonstrated that AML-sEVs downregulated the key proteins that are important for normal haematopoiesis in BM-MSCs. In addition, we revealed that AML-sEVs significantly reduced the differentiation of BM-MSCs to osteoblasts. Next, LC-MS/MS elucidated that various proteins, including YBX1 were upregulated in both AML-sEVs and BM-MSCs treated with AML-sEVs. Clinically relevant, we found that YBX1 is considerably upregulated in most paediatric AML patient-derived sEVs compared to healthy controls. Interestingly, sEVs isolated after the downregulation of YBX1 in AML cells remarkably rescued the osteoblastic differentiation of BM-MSCs. Altogether, our data demonstrate for the first time that YBX1 containing AML-sEVs disrupt the normal function of bone marrow microenvironment by reducing the osteogenic differentiation of BM-MSCs.

0044 Dual targeting of FLT3-ITD and CLK by screened donated chemical probes in acute myeloid leukemia

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Despite aggressive treatment regimens, the survival rate for children with acute myeloid leukemia (AML) remains dismal. The urgent need for novel therapeutic strategies has led to great interest in targeted therapies. To explore the option of different targeted therapies, we conducted a screening of 105 small molecules AML cell lines and patient-derived blasts of childhood AML. Here, we discovered that inhibition of Cdc2-like kinases (CLKs) effectively reduced cell viability and induced cell cylce arrest, as well as apoptotic cell death in both, cell lines and patient-derived AML cells. Further characterization of a distinct

CLK-inhibitor revealed a predominant efficacy in FLT3-ITD mutated AML, hinting at an intriguing off-target effect on FLT3-ITD. Strikingly, this dual therapeutic approach shows great promise in FLT3-inhibitor resistant AML cells. More interestingly, a CRISPR Cas9-dropout screening revealed, that a combination with CDK6-inhibition enhances the effect of the compound. Based on these results, we believe that this compound, especially in combination with inhibitors of CDK6, might represent a promising therapeutic strategy for FLT3-ITD AML

0045 Exploring Synergistic Approaches to Enhance Blinatumomab's Efficacy in Acute Lymphoblastic Leukemia

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Resistance to blinatumomab (CD19-CD3 BiTE) is a significant obstacle in the treatment of B-cell precursor acute lymphoblastic leukemia (BCP-ALL), prompting the investigation of novel drug combinations. We aimed to generate an "in vitro" model capable of identifying synergistic combinations to enhance blinatumomab's efficacy in BCP-ALL treatment. Drug response profiling used annexin V/propidium iodide/CD3 staining and flow cytometry analysis in 28 patient samples and 4 cell lines. Co-cultured with healthy donor T-cells, samples were treated for 24h with blinatumomab and/or other inhibitors. Differential sensitivity to blinatumomab was observed among patient samples and cell lines. Idelalisib (tyrosin kinase inhibitor; TKi) combined with blinatumomab exhibited potential antagonistic effects. Birinapant (SMAC mimetic) and venetoclax (BCL2 inhibitor) demonstrated increased efficacy in BCP-ALL cell lines, displaying synergistic potential with blinatumomab. Our findings support TKi and SMAC mimetics' immunomodulatory effects, in accordance to prior anti-CD19 CAR T cell reports. Venetoclax emerges as a promising candidate for combination therapy against blinatumomab resistance.

0046 MiR-181a in Pediatric Acute Lymphoblastic Leukemia of the Central Nervous System

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Understanding the molecular biology of pediatric central nervous system (CNS) acute lymphoblastic leukemia (ALL) is crucial for developing new CNS-directed therapies. Recently, we identified vascular endothelial growth factor A (VEGF) as a regulator for CNS ALL aiding ALL transmigration into and adaptation to the nutrient-low CNS niche. In cerebrospinal fluid of patients miR-181a was reported to be a biomarker for CNS ALL and to regulate VEGF expression in chondrosarcoma. Hence, we evaluated the association of miR-181a and VEGF in CNS ALL. We analyzed VEGF and miR-181a expression in 22 patient-derived xenograft samples. VEGF was significantly upregulated in CNS- compared to bone marrow-derived ALL cells. Despite no differential expression of miR-181a, a significant correlation with VEGF expression was found. MiR-181a mimics had no effect on transmigration, yet VEGF protein increased significantly exclusively in low-nutrient conditions. In sum, miR-181a regulates VEGF expression in ALL cells cultured in conditions mimicking the CNS milieu. MiR-181a target genes interposed to VEGF require identification with the possibility to establish novel CNS-directed druggabilities.

0047 siRNA Delivery for the Treatment of RUNX1::ETO Driven Acute Myeloid Leukemia.

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The RUNX1::ETO fusion gene is present in 15 % of all AML cases. We designed a siRNA approach for specific targeting of this fusion gene and are developing lipid nanoparticles (LNPs) for efficient in vivo siRNA delivery. To further improve physicochemical, pharmacokinetic (PK) and -dynamic (PD) properties, we evaluated the impact of novel helper and stealth lipids on LNP size, charge, shelf life and ex vivo efficacy. Replacement of cholesterol by β-sitosterol improved knockdown efficacy by more than 5-fold but impaired LNP stability. We restored the stability by including sphingomyelin and tweaking the molar lipid ratios. Furthermore, the replacement of PEG with the biodegradable polysarcosine allowed increasing the stealthiness without compromising activity. When decorated with a ligand for VLA-4 recognition, expressed on all hematopoietic cells, the optimized formulations achieved knockdowns in the range of 70 % after a single dose in patient-derived AML cells. The in vivo studies are ongoing to examine the PK and PD properties of the novel LNP formulations. Our results emphasize the importance of fine-tuning the LNP composition to achieve balance between stability and effectiveness

0048 CD19-CAR-NK cells show ability to initiate diverse mechanisms of cancer cell death in hematological malignancies

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DOI 10.1055/s-0044-1786599
Umbilical cord blood-derived CD19-CAR-NK cells demonstrated prolonged overall survival without severe side effects in first clinical trials against CD19 + lymphoid malignancies. However, specific underlying mechanisms of CAR-NK cytotoxicity needs to be unraveled. Here, we aim to investigate the different pathways leading to efficient tumor cell death with a focus on serial killing strategies induced by healthy donor-derived primary CD19-CAR-NK cells using live cell imaging. With the fluorescence-based NALM-6 pCasper system, we can investigate killing trajectories as well as discriminate between apoptotic and necrotic tumor cell death. We found an increased level of apoptosis and direct necrosis of tumor cells induced by CD19-CAR-NK cells compared to non-transduced NK cells. Using high resolution microscopy, serial killing by a single CAR-NK cell attacking multiple hematological tumor cells was observed. In addition, the impact of different key targets will be assessed using inhibitory antibodies to gain a deeper understanding of the underlying mechanisms of CAR-NK cell cytotoxicity. The ability of serial killing could highlight the NK cell inherent strong anti-tumor potential.

0049 Pharmacotyping identifies venetoclax as effective salvage therapy in primary refractory MLLT10:PICALM acute leukemia

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Despite cure rates exceeding 90 %, relapsed/refractory B-cell precursor acute lymphoblastic leukemia (BCP-ALL) remains a clinical challenge. Previously, we established an ex vivo drug response profiling (DRP) in which the leukemia cells are exposed to increasing concentrations of different established and experimental drugs allowing estimation of half-maximal effective concentrations (EC50 values). Here, we aimed to identify effective, clinically applicable drugs in the case of a patient with primary refractory BCP-ALL at the time of induction failure. DRP confirmed the clinically observed resistance to the induction che-

motherapy drugs asparaginase, vincristine, daunorubicin and prednisone. However, excellent sensitivity to the BCL-2 inhibitor venetoclax was identified. Treatment of the patient with venetoclax together with low-dose chemotherapy led to induction of complete remission with nearly undetectable MRD levels, demonstrating a successful bench-to-bedside translation. Further molecular analysis revealed high gene and protein expression of the target molecule BCL-2. No recurrent genetic abnormalities were found, however, a MLLT10::PICALM fusion was identified by RNA-Seq.

0050 Single cell RNA sequencing unravels targetable interaction axes in B-cell acute lymphoblastic leukemia

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DOI 10.1055/s-0044-1786601

The stromal compartment of the bone marrow micro-environment supports leukemogenesis through secretion of soluble factors, cell-cell interactions and transfer of metabolites and organelles via nanotubes. However, the communication axes necessary for leukemia onset and progression remain unclear. By performing single-cell RNA sequencing on patient-derived bone marrow mononuclear cells on pediatric B cell acute lymphoblastic leukemia patients (n = 9), 11 main cell clusters were identified. Spanning leukemic cells, MSCs, erythroblasts, CD4 + T cells, CD8 + T cells, regulatory T cells, NK cells, myeloid progenitors, monocytes, dendritic cells, and naive B cells. Ligand-receptor interaction analysis identified stromal cells as having the strongest leukemia cell-cell interaction score. Key molecular signaling pathways were indicated for the interaction between stromal cells and leukemic cells, such as the Osteopontin-CD44 axis and CXCL12-CXCR4 pathway. These interactions present interesting leads for further investigation and possible targets for new therapeutics.

0051 HDAC7 induction prevents immune escape in high-risk infant pro-B-ALL

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Infant pro-B acute lymphoblastic leukemia (pro-B-ALL) with t(4;11) rearrangement presents an aggressive phenotype and very poor response to conventional chemotherapy. Moreover, immune escape mechanisms such as lineage switch involve the loss of B cell markers, leading to CD19-CAR-T therapy failure. Our previous findings have demonstrated that histone deacetylase HDAC7, a key factor in B cell differentiation, is underexpressed in t (4;11) pro-B-ALL. However, the precise induction of HDAC7, mediated by a previously defined drug combination, blocks t (4;11) pro-B-ALL cells from acquiring myeloid cell features and induces acquisition of lymphoid genes such as CD19, both in vitro and in vivo. In parallel, ex vivo culture of t (4;11) pro-B-ALL primary cells has revealed the presence of a subpopulation with low CD19 expression. Remarkably, the use of HDAC7-inducing therapy shifts this CD19 low cells into CD19 high population, thus reducing the ration of CD19 low cells, most likely to undergo lineage switch and display immunotherapy resistance. Therefore, HDAC7 induction is a promising therapeutic strategy to improve response of t (4;11) pro-B-ALL patients to CD19 CAR-T cell therapy.

0052 Exploring the translational potential of EZH2-controlled fetal gene signature in AML

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Enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase and the main enzymatic subunit of the polycomb repressive complex 2 (PRC2), which regulates gene expression through tri-methylation of histone 3 at lysine 27. Around 2-13 % of acute myeloid leukemia (AML) patients have mutated EZH2. Our studies highlighted that loss of EZH2 reactivates fetal gene sets, whose expression is high during embryogenesis and tumorigenesis but silent in healthy adult cells. Reactivation of these fetal gene signatures was shown to be a leukemogenic driver event in mouse models and reactivation was found to be overrepresented in patients with EZH2 and PRC2 mutations. Noteworthy, this was observed mainly in high-risk AML patients. By implementing CRISPR-Cas9 screens, we now aim to identify reactivated fetal genes that are therapeutically exploitable AML weaknesses. Moreover, we aim to identify additional epigenetic regulation of the fetal genes in AML using state-of-the-art CRISPR interference technology. Our results will provide deeper insight on the molecular mechanisms of EZH2 and its behavior in malignancies that could contribute to the development of targeted therapies for AML patients.

0053 An innovative tailored CAR T cell-redirecting immunotherapy for the treatment of metastatic and refractory Ewing Sarcoma

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Ewing Sarcoma (ES) stands as the second most common bone and soft tissue sarcoma affecting children and young adults. Limited response rates to current multimodal therapies and the lack of actionable targets contribute to dismal long-term survival in patients with metastatic/refractory disease. This highlights the urgent need for developing novel therapeutic approaches. Immunotherapy employing chimeric antigen receptor (CAR)T-cells directed against a tumour-associated antigen (TAA) is a promising therapy with a safe and efficient profile in B-cell malignancies. However, the development of efficient and safe CAR T-cells for the treatment of ES remains challenging due to i) the low abundance of specific and safe TAAs, ii) and the presence of an immunosuppressive tumour microenvironment (iTME) that limits CAR T-cells function. Here, we present an efficient CAR T-cell approach targeting a specific ES-associated antigens with limited expression in healthy tissues. We functionally validated its specificity and safety profiles in robust in vitro and in vivo assays using ES cell lines, primary cells, 3D sarcoma spheroids models which mimic the ES-iTME, and patient-derived animal models.

0054 Combination of BH3 mimetics with NK cell-based immunotherapy as a potential novel treatment option for pediatric sarcoma

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DOI 10.1055/s-0044-1786605

Rhabdomyosarcoma (RMS) is the most prevalent malignant soft tissue cancer in children. Despite intensive therapy regimens, survival rates are low, and better treatment options are urgently required. To discover improved treatment alternatives, in vitro models accurately reflecting tumor biology are necessary. Therefore, we established a pipeline to develop primary patient-derived cells as new models for pre-clinical drug and immunotherapy testing. As proof of concept, we tested allogenic Natural Killer (NK) cell attack and BH3-mimetics in primary 3D spheroids (n = 6) as well as 2D cultures. Initial results show that RMS are sensitive to NK cell attack as well as selective BH3-mimetics targeting BCL-XL. To further expand these findings, we are optimizing culture conditions to perform large-scale drug screening approaches as well as mechanistic studies in RMS. Taken together, we validated the use of primary cell-derived tumor spheroids as models of sarcoma and established a platform for the development of novel therapeutics.

0055 Interplay between macrophages and AML blast in pediatric acute myeloid leukemia

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Patients suffering from AML still lack significant benefits from immunotherapeutic approaches. One reason for this is an incomplete understanding of the AML microenvironment (ME), and here in particular of the role of components of the innate immune system. In this study, we focus on the characterization of macrophage phenotypes and their respective cellular interaction with leukemic and non-leukemic cells in pediatric AML bone marrow. In two independent pediatric AML cohorts, we identified M2 macrophages as the main residents in AML bone marrow with only a small fraction of cells of the M1 phenotype. Co-culture experiments revealed that M2c and M2d macrophages support AML cell proliferation, while M1 macrophages inhibit AML cell growth. However, M1 macrophages lost their anti-tumor activity after 6 days of coculture. This is associated with reeducation of M1 macrophages to an anti-inflammatory phenotype and function by AML blasts. We predict that an understanding of the interaction between AML blasts and bone marrow macrophages will identify novel targets for reversing the immunosuppressive ME in pediatric AML and enhance T/NK-cell engaging immunotherapies.

0056 The testicular niche – heterogeneity in leukemic cell adaptation to the new immunemicroenvironment

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Approximately 15 % of patients with a B-cell precursor acute lymphoblastic leukemia (B-ALL) relapse, with the testis being the second most common extramedullary site of relapse in boys. Mechanisms behind blast survival in the testis remain unclear. We conducted a multi-modal comprehensive molecular analysis, focusing on the comparative transcriptomic landscape of testicular vs bone marrow relapses and the spatial architecture of tumor immune microenvironment (TiME). We performed high dimensional bulk RNA-seq analysis from a cohort of 38 relapsed pediatric ALL samples, identifying distinct gene expression signatures and explored the spatial composition of the TiME in the invaded testis at single-cell resolution with Imaging Mass Cytometry.
Our findings indicate that M2 macrophages and different T cell subtypes dominate the microenvironment, albeit with inter-patient heterogeneity with distinct infiltration patterns ranging from immune deserts to tertiary lymphoid-like structures. Ongoing spatial transcriptomics and scRNA-seq experiments will be instrumental to gain a better understanding of the interaction between B-ALL cells and immune / non-immune cells of the testicular niche.

0057 ABC transporters modulate the response of AML cells to Menin-MLL inhibitors

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Multiple high-risk subtypes of Acute Myeloid Leukemia (AML) require the Menin-MLL1 interaction to sustain leukemogenic gene expression. Small-molecule inhibitors of the Menin-MLL1 interaction, such as Revumenib and Ziftomenib have shown promising efficacy in preclinical models and first clinical trials. However, there is a lack of biomarkers that could predict the response to Menin-MLL1 inhibitors. Furthermore, the cellular pharmacokinetics of accumulation, metabolism and excretion of Menin-MLL inhibitors are mostly unknown. ATP-binding cassette (ABC) transporters play important roles in the metabolism of anticancer drugs, but it was not known if they modulate the activity of Menin-MLL inhibitors. Using systematic CRISPR/Cas9-enabled functional genomics, pharmacological and cell biological approaches, we identified several candidate ABC transporters whose modulation changes the response of AML cells to Menin-MLL inhibitors. Future investigation of their role in modulating the response and resistance of AML to Menin-MLL inhibitors will reveal important insights into intracellular drug metabolism and nominate novel functional biomarkers that predict drug response.

0058 A Novel Bone Marrow Organoid Platform for Studying Hematological Malignancies

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DOI 10.1055/s-0044-1786609
Maintaining and expanding primary leukemic cells in vitro poses considerable challenges due to their low viability and rapid differentiation rates. Despite recent progress, current 2D co-culture systems lack complexity and miss out on crucial interactions with the bone marrow niche. Recently published bone marrow organoids (BMOs) promise the study of leukemic cell interactions with their 3D bone marrow microenvironment.
We generated BMOs from human induced pluripotent stem cells (iPSCs) and assessed them via flow cytometry and confocal microscopy. Our analysis revealed successful differentiation into mesenchymal stem cells, cell types of myelopoietic bone marrow and the generation of complex vascular networks, containing hematopoietic cells. Currently we are investigating whether BMOs allow for engraftment of primary acute myeloid leukemic cells and examine BMO hematopoiesis in more detail by analyzing key marker expression (e.g. HOXA9). BMOs offer a physiologically relevant environment which could enhance our understanding of leukemia pathogenesis and facilitate the development of more effective therapeutic strategies, while surpassing conventional 2D platforms and PDX models.

0059 Targeting ATRX-Deficient Hepatoblastoma

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Hepatoblastoma (HB) is the most common pediatric liver tumor. HB is genetically a very simple cancer with an average of only 3.4 mutations per tumor. New exome sequencing data on a larger series of aggressive cases revealed new

candidate genes associated with an increased mutation number and chromosomal instability. One prominent candidate is the alpha thalassemia/mental retardation syndrome X-linked (ATRX) gene, for which loss of function is known to trigger rampant signs of genomic instability.
To investigate the role of ATRX mutation in HB, we utilized the CRISPR-Cas9 system to functionally inactivate the ATRX gene in HB cell lines. Compared to wild-type cells, ATRX-deficient cell lines show reduced proliferation and compromised DNA repair capacities. RNA sequencing of mutant cells has provided insight into the molecular mechanisms of ATRX deficiency in HB. Using bioinformatics tools, this data is used to predict drugs specifically targeting ATRX-deficient HB. This study will contribute to our understanding of hypermutated aggressive HB and may lead to treatments specifically tailored to these patients.

0060 IL-7R immunotherapy combined with chemotherapy effectively reduces xenograft acute lymphoblastic leukemia (ALL)

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ALL arises from the uncontrolled proliferation of precursor B or T cells (BCP- or T-ALL). Current treatment protocols obtain high cure rates in children but are based on toxic polychemotherapy. Novel immunotherapies are needed, especially in T-ALL, which can also be treated with venetoclax (VEN). The IL-7Rα (CD127) is highly expressed on BCP- and T-ALL cells and represents a promising target in both entities. We found that antibody-based immunotherapy (IT) directed against CD127 can be highly effective in minimal residual disease (MRD) in patient-derived xenograft (PDX) models of BCP- and T-ALL. Of note, this effect was especially pronounced after precedent chemotherapy ("post-chemo MRD"). In a phase2-like preclinical PDX-study modelling overt leukemia, CD127-IT was equally effective as induction-like polychemotherapy. Importantly, the combination of both led to MRD-negativity in 5/9 PDX-models. Moreover, Bcl2-inhibition with VEN enhanced CD127-IT efficacy in vitro. Our data suggest CD127-IT as a novel immunotherapeutic strategy for the treatment of BCP- and T-ALL, particularly in relapsed/refractory disease and in combination with chemotherapy warranting clinical investigation.

0061 EGFR/EGFRvIII-targeted CAR-NK cells as a promising allogeneic cell therapy for the treatment of solid tumors

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DOI 10.1055/s-0044-1786612
Natural killer (NK) cells engineered with chimeric antigen receptors (CARs) have emerged as a promising alternative to CAR-T cell immunotherapies. In clinical trials, CAR-NK cells have demonstrated efficacy against hematological malignancies, while targeting solid tumors remains challenging. Here, we investigated the antitumor activity of healthy donor derived primary CAR-NK cells engineered to express a cetuximab-based CAR (225.28.z) targeting both, epidermal growth factor receptor (EGFR) and the tumor-specific variant EGFRvIII for the treatment of rhabdomyosarcoma (RMS) and glioblastoma (GB). Lentivirally transduced NK cells showed stable CAR expression in 50-60 % of cells and significantly enhanced cytotoxicity against RMS and GB cell lines in vitro in short-term 2D cultures and long-term assays with tumor organoids. In vivo, we observed delayed tumor growth in a subcutaneous (s.c.) GB xenograft model after a single intravenous injection of CAR-NK cells. Likewise, combining repeated CAR-NK cell injections with radiotherapy in an s.c. RMS model reduced tumor growth, which may provide a promising avenue for translating this approach into early clinical testing.

0062 Exploring BCL-xL inhibition for the treatment of pediatric acute megakaryoblastic leukemia

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BCL-2 like proteins play an important role in promoting leukemia and in developing resistance to chemotherapeutics. Accordingly, BCL-2 inhibition is effective in the treatment of adult acute myeloid leukemia (AML). However, in acute megakaryoblastic leukemia, including myeloid leukemia in Down-Syndrome (ML-DS), BCL-xL is more important for controlling mitochondrial pathway of apoptosis. Indeed, ML-DS patient cells showed better in vitro response to BCL-xL blocker Navitoclax than to BCL-2 blocker Venetoclax. But Navitoclax alone does not improve survival in vivo. To further explore and enhance the effectivity of BCL-xL inhibition as therapeutic option, we performed CRISPR/Cas9 screens aiming to find genes promoting or interfering with leukemia proliferation when Navitoclax is applied. We screened genes differentially expressed between Navitoclax responders/ non-responders based proteomic data as well as on genes targeted by FDA approved drugs. We aim to identify potential drug targets sensitizing BCL-xL inhibition and to discover resistance mechanisms providing new avenues for exploring therapeutic options for various malignancies including pediatric acute megakaryoblastic leukemia.

0063 Deciphering the Epigenetic Landscape of Relapsed Myeloid Leukemia in Children with Down Syndrome

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Children with Down syndrome (DS) are at high risk for myeloid leukemia (ML-DS), facing excellent prognosis in first line therapy and poor outcomes upon relapse. Mutation-focused diagnostics fall short in explaining this apparent paradox. This study posits epigenetic mechanisms as potential key drivers of relapse, aiming to unravel these processes for better therapeutic strategies. We analyzed the epigenetic and transcriptomic profiles of initial and relapse ML-DS samples, utilizing patient-derived xenografts (PDX) for comprehensive in vitro and in vivo examinations. Techniques like CUT&RUN and CUT&Tag were used to reveal distinct histone modifications and transcription factor chromatin engagements linked to relapse, indicating a shift in chromatin accessibility and transcription factor activity that may underpin therapy resistance. Functional profiling of relapse PDXs highlighted chromatin modifications and transcriptional shifts central to disease recurrence, paving the way for targeted treatments. Our findings suggest that epigenetic regulation plays a crucial role in ML-DS relapse, offering new avenues for therapeutic intervention to enhance survival in this high-risk group.

0064 Probing the non-coding cancer transcriptome for context-dependent vulnerabilities

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Nowadays, about 20000 protein-coding genes and their corresponding mRNAs have been discovered. In comparison to these coding RNAs, there are far more so-called non-coding RNAs (ncRNAs), which fulfill their main functions without coding for proteins. In fact, about 76 % to 97 % of the human genome encodes for these RNA molecules. The biological importance of this heterogeneous

group consisting of many different RNA entities such as circRNAs, lncRNAs or miRNAs has become increasingly apparent. Since ncRNAs oftentimes serve as important regulators of gene expression and genome organization, it is not surprising that a dysregulation of these entities can lead to human diseases such as cancer. This contribution to the onset of disease highlights the potential of ncRNAs as therapeutic targets. To determine potential dependencies of cancerous cells on ncRNAs, we are performing a CRISPR screen targeting both coding and non-coding RNAs in several cell lines. DNA sequences coding for ncRNAs are often found within protein-coding genes, which renders the traditionally used Cas9 nuclease less suitable for the specific perturbation of ncRNAs. We are therefore utilizing Cas13 for this application.

0065 Cdk6's functions are critically regulated by its unique C-terminus

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The family of cyclin-dependent kinases (CDKs) comprises more than 20 serine/threonine kinases functioning in diverse cellular processes including cell-cycle progression and transcriptional regulation. Dysregulation of CDKs is directly linked to tumorigenesis, e.g. leukemic cells highly depend on CDK6, a pivotal component in the cell cycle machinery which also acts as a transcriptional regulator. As the C-terminus is the region which differs the most between CDKs, it is of high importance to clarify its influence on the protein functions. Employing a comprehensive approach involving proteomic analysis and computational modelling we demonstrate that the C-terminus of CDK6, is indispensable for protein flexibility and binding to its key interaction partners, cyclin D, p27Kip1 and INK4 proteins. Expression of a C-terminally truncated Cdk6 in Cdk6-/- leukemic cells, reveals reduced nuclear translocation and chromatin interaction accompanied with a failure to induce proliferation and disease promotion. We demonstrate that the C-terminus is a unique and essential part of the CDK6 protein, regulating interaction partner binding and therefore CDK6's functionality.

0066 Validation of ICH Q2(R2) compliant flow cytometry-based Quality Control for the characterization of CAR-NK cell products

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In the last decade, cellular immunotherapies using genetically engineered cells to express chimeric antigen receptors (CAR) have made tremendous progress. In order to safely apply CAR-NK cell products in the context of a clinical trial or further as an ATMP, GMP-compliant manufacturing and Quality Control (QC) is essential. Regulatory requirements for QC of ATMPs are extensive and have to include test specifications such as identity, purity, impurities, quantity, potency and safety. By developing 3 robust 13-color single-platform immunophenotyping panels on the DxFLEX cytometer we are able to determine multiple test specifications at once while analyzing cytotoxicity and fitness of the cells. Killing-superiority of CAR-NK cells over untransduced cells is demonstrated also by a flow-based potency assay. The optimized quality control is currently being validated following the guidelines of the International Council for Harmonisation (ICH) Q2 (R2), addressing validation parameters such as specificity, accuracy, stability and robustness. This ensures precise description of the pharmacologically active and contaminating cell populations for a safe CAR-NK cell product application.

0067 The impact of extrachromosomal MDM2 amplifications on tumor heterogeneity and treatment resistance

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Despite advances in precision oncology, persisting treatment resistance necessitates a better understanding of therapy response mechanisms. In various tumours, extrachromosomal amplification of oncogenes accelerates intratumoral heterogeneity development, for example through random segregation during cell division due to a lack of centromeric structures. The oncogene murine double minute 2 (MDM2), which inhibits tumour suppressor p53, is amplified on extrachromosomal DNA (ecDNA) in several tumour diseases. To show how MDM2 ecDNA contributes to intercellular heterogeneity, we used neuroblastoma cell lines with extrachromosomal or linear MDM2 amplification combining fluorescence in-situ hybridisation with immunofluorescence for MDM2, p53, and cell proliferation markers. Upon exposure to doxorubicin or to MDM2 inhibitor Nutlin-3a, only cell lines carrying MDM2 ecDNA showed dynamic modifications in MDM2 copy number, measured by FISH. In cell lines bearing additional oncogenes on separate ecDNA species, co-depletion or co-enrichment was observed. Our study underscores the importance of considering ecDNA-driven processes when employing cytotoxic and targeted therapeutic strategies.

0068 Preemptive Targeting of Preleukemic Cells in Down Syndrome with Pathway-Directed Therapies

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Preventing myeloid malignancies at a preleukemic stage remains significant. Myeloid leukemia in Down syndrome (ML-DS) and the precedent TAM present genetically simple models for studying leukemia progression. TAM is marked by prenatal origins, trisomy 21, mutations in GATA1 and a 20 % chance of progressing into ML-DS. Our aim is to establish preemptive treatments to prevent progression to leukemia. We employed CRISPR/Cas9 screens on a murine fetal hematopoietic stem/progenitor model integrated with bioinformatic analysis. Utilizing a sgRNA library targeting genes associated with FDA-approved drugs, we are proposing that gene knockouts mimic the therapeutic effects of drugs targeting the same proteins. The investigation underscored the reliance of TAM/ML-DS on the purine biosynthesis pathway. Remarkably, our data showed high specificity in targeting the pathway, validated by the effective treatment of patient-derived TAM blasts. Treatment was found to induce cell cycle arrest and apoptosis. Prolonged exposure further promoted megakaryocytic differentiation. We are proposing a novel treatment to eliminate preleukemia and prevent the transition to overt leukemia.

0069 Targeting the non-coding and stem cell signature in childhood acute myeloid leukemia

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DOI 10.1055/s-0044-1786620

Despite an overall survival rate of 70-75 %, pediatric acute myeloid leukemia (AML) continues to pose significant clinical challenges. Our study embarked on identifying new therapeutic targets through a comprehensive comparative RNA sequencing analysis between pediatric AML patients and healthy individuals. This exploration revealed unique expression patterns in 1679 coding genes and, importantly, 696 long non-coding RNAs (lncRNAs), highlighting the critical role of non-coding genes in AML's development. Employing complementary CRISPR/Cas9 and inhibition screenings, we identified key genes integral to AML pathology for deeper examination. Subsequent validation through knockout assays and qPCR unveiled a previously unrecognized significance of the heme biosynthesis pathway across various AML types. Our findings not only emphasize the importance of deregulated metabolic pathways in AML but also open new pathways for therapeutic development, underscoring the untapped potential of targeting metabolic and epigenetic mechanisms in combating this malignancy.

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

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DOI 10.1055/s-0044-1786621

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 241 pediatric cases plus 158 healthy donor samples, representing the normal hematopoiesis. Integrating genomic, transcriptomic, and epigenetic data with clinical insights, our work advances AML subtype classification and elucidates the impact of somatic mutations on prognosis, especially impacting overall and event-free survival. This integration highlights the importance of mutational networks over individual aberrations in disease progression and therapeutic response. Through Multi-Omics Factor Analysis (MOFA), we uncover intricate molecular interactions that define AML's heterogeneity. This provides deep insights into AML's pathogenesis identifying therapy targets. Our findings underscore the benefits of multi-omics integration in enhancing disease understanding, improving classification and prognosis, and paving the way for precision oncology in AML. The HemAtlas 2.0 project highlights the potential of combining diverse omic layers and clinical data to refine patient care strategies.

0071 Single Cell Sequencing to Characterize the Bone Marrow Microenvironment in KMT2A Rearranged Pediatric AML

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Despite intensive chemotherapy, approximately one third of pediatric AML (pAML) patients relapse, while the mechanisms behind relapse are poorly understood. One reason for this is the 'hijacked' bone marrow (BM) microenvironment by the leukemia. Not only do leukemic cells alter the function of T-cells, but increasing evidence points to the importance of innate immune cells and stromal cells as well in creating an immune suppressive and drug resistive environment. In pAML this is poorly characterized. With this aim, we are generating single cell RNA sequencing data on KMT2A rearranged pAML patients, at diagnosis, after the first induction, before the last therapy course and at relapse. To study the stromal compartment, we developed a FACS strategy to enrich this low abundant cell type. We will characterize the non-hematopoietic BM cells during treatment timepoints, study altered pathways and cell-cell interactions. This will be further investigated by spatial transcriptomics and proteomics with bone biopsies and in vitro co-culture assays. We expect to see more dysfunctional immune cells and leukemic supporting stromal clusters in relapse compared to remission patients.

0072 Distribution of BCR::ABL1 transcript types and response to therapy in pediatric patients with chronic myeloid leukemia

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In the management of chronic myeloid leukemia (CML) in pediatric patients, achieving treatment-free remission has become a primary objective to reduce the side effects of tyrosine kinase inhibitor (TKI) therapy, such as growth retardation. Furthermore, identifying risk factors for inadequate response to TKIs is an ongoing goal in CML.

Our aim was to assess whether the BCR::ABL1 transcript type impacts therapy response in pediatric patients with CML. Conflicting data exists regarding the potential risk factor of the e13a2 transcript in adult CML. Using an optimized droplet digital PCR detection assay with reduced technical bias, we identified significantly more patients expressing two BCR::ABL1 transcript variants simultaneously than previously described. Parallel monitoring of both transcripts in a single patient allows for a consistent evaluation of their differing therapy responses within the same biological system. Based on these results and our additional data from in vitro experiments, we can for the first time explicitly rule out a differential response to TKI-treatment between the two primary BCR::ABL1 transcript variants in CML.

0073 Unraveling the role of PDHA1 in neuroblastoma metastasis

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Neuroblastoma (NB) is the most common extracranial solid tumor in children. Over 60 % of all diagnosed NB are metastatic. In other cancers, pyruvate dehydrogenase (PDHA) increases metabolic flexibility and promotes metastasis. However, the role of PDHA1 in NB progression is still unknown. Bioinformatic analysis of patient datasets and ChIP-Seq analysis revealed that PDHA1 is a target of the transcription factor and important clinical biomarker MYCN. PDHA1 expression is markedly associated with poor survival and significantly correlates with metastasis in NB independent of MYCN. Altogether, this identifies PDHA1 as a marker for poor prognosis in NB. Moreover, we evaluated whether PDHA1 has an enhancing or suppressing effect on NB metastasis and which molecular mechanisms mediate this effect. Complete PDHA1 knockout (KO) in NB cells decreases in vitro some but not other key steps of the metastatic cascade. This functional PDHA1 KO shifts the metabolic activity towards aerobic glycolysis and decreases mean basal respiration. In vivo analyses of the role of PDHA1 in NB metastasis are ongoing. Taken together, we provide preliminary evidence that PDHA1 may be involved in metastatic NB.

0074 Primary CAR-NK cells targeting B7-H3 as a novel experimental immunotherapeutic strategy against glioblastoma

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Glioblastoma (GB) is the most common and aggressive brain tumor in adults with a 5-year survival rate of only 10 %. Conventional treatment regimens include surgery, radiation and chemotherapy. The ongoing CAR2BRAIN trial (NCT: 03383978) is currently evaluating the efficacy of the genetically modified natural killer cell line NK-92 (NK-92/5.28.z) targeting HER2 for the treatment of patients with recurrent HER2-positive GB. In this project, we address genetically engineered primary NK cells for treatment of GB, which are characterized by high intrinsic cytotoxicity and prolonged persistence. To achieve specificity, B7-H3 was chosen as a pan-cancer target highly expressed in many solid tumors, including GB. B7-H3-CAR-NK cells show stable CAR expression (55–75 %) and enhanced anti-tumor efficacy compared to non-transduced NK cells in 2D and 3D in vitro models. Further, we will investigate the receptor and cytokine repertoire of B7-H3-CAR-NK cells before and after tumor contact to gain a deeper understanding of the underlying mechanisms of CAR-NK cell-mediated cytotoxicity.

0075 Exploring the immune microenvironment dynamics in AML progression via single cell analysis

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DOI 10.1055/s-0044-1786626

The KMT2A-MLLT3 is the most prevalent KMT2A alteration in pediatric AML patients. Here we aimed to investigate the interactions between leukemic and immune cells in a mouse model that maintains the natural bone marrow (BM) environment. We transplanted naïve BM cells from inducible iKMT2A-MLLT3 (CD45.2 +) transgenic mice into immunocompetent (CD45.1 +) recipient mice, followed by activation of the fusion via doxycycline (Dox) resulting in myelomonocytic AML in 20 % of the recipients. We assessed disease initiation and progression by analyzing histology, immune cell populations by flow cytometry and transcriptomic analysis. In particular, Cite-seq single-cell analysis of BM cells from transplanted mice across early, late and disease-free stages enabled us to identify transcriptomic changes within immune cell populations at different stages of the disease. Notably, early-stage T cell clusters exhibited increased expression of activation genes compared to exhaustion-related genes during leukemia progression. Ongoing investigations aim to dissect the epigenomic factors responsible for evading immune surveillance and promoting disease progression.

0076 Regulatory mechanisms of the DLK1-DIO3 locus in the hematopoietic system and pediatric acute myeloid leukemia

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DOI 10.1055/s-0044-1786627

Non-coding RNAs (ncRNAs) recently emerged as central regulators of chromatin and gene expression, posing a novel window for targeted therapies in pediatric acute myeloid leukemia (AML). We established a comprehensive ncRNA expression atlas for the hematopoietic system including 46 pediatric AML patient samples and discovered genes within the DLK1-DIO3 (DD) locus to be highly expressed in hematopoietic stem cells (HSCs) and megakaryocytes. Interestingly, patients with acute megakaryoblastic leukemia (AMKL) were dependent on high expression of the members of the DD locus.
We employed a range of methods to investigate the regulatory mechanisms governing the DD locus. By mapping histone modifications, we identified critical enhancers. We conducted CUT&RUN assays to detect the binding sites of GATA1, within AMKL cell lines and found that both GATA1 andGATA1s, bind to multiple genomic regions upstream of DLK1 and MEG3. Using CRISPR/Cas9 to target the binding sites of GATA1/GATA1s led to a reduction in cell proliferation and decreased expression of DLK1 and MEG3 in AML cell lines. Our study is the first step in understanding the regulation of the DD locus in AML and hematopoiesis.

0077 Novel treatment approaches for pediatric high-grade gliomas with MYCN amplification

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Tumors of the central nervous system (CNS) rank as the second most common group of malignant diseases in children, with gliomas comprising about 50 % of pediatric CNS tumors. Among these, highly malignant gliomas, including the recently recognized entity of MYCN-amplified high-grade gliomas (HGG-MYCN), exhibit particularly aggressive growth patterns. The prognosis for patients with HGG-MYCN is poor, with a median survival of about 14 months. Currently, targeted therapies are lacking.
A transgenic mouse model generating forebrain tumors comparable to human HGG-MYCN has been established successfully. Paired with a human tumor cell line, this model enabled high-throughput drug screening, leading to the identification of potential therapeutic agents (Schoof et al., 2023). In this study, we aim to validate the efficacy of identified compounds. Promising candidates, including Irinotecan, Doxorubicin, and Alisertib, were validated through in vitro assays. Concentration-response curves indicated a selective efficacy against murine and human HGG-MYCN tumor cells.
Our findings suggest potential effective treatment options for HGG-MYCN, which are more effective than the current therapy.

0078 Modulating ubiquitin signaling to control cell death and necroinflammation in patient-derived human mammary organoids

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Breast cancer (BC) is the most common cancer in women. In luminal BC, high NF-kB signaling causes programmed cell death (PCD) resistance and tumor survival. NF-kB signaling was targeted through inhibiting E3 ligases cIAP1/2 and XIAP by SMAC mimetics in 3D patient-derived human BC organoid (hMOs). SMAC mimetics induced PCD without ectopic TNF-a. Next, apoptosis resistance was modeled by applying a pan-Caspase inhibitor. Cell viability showed a donor-dependent decrease upon single and combination treatment. Immunofluorescent microscopy and Western Blot confirmed the onset of apoptosis and necroptosis in these respective treatments. In necroptotic hMOs, we identified an upregulation of inflammatory cytokines in gene expression and protein secretion. Notably, inhibition of LUBAC by small molecule HOIPIN-8 reversed the inflammatory signaling. scRNAseq investigated the influence of cell clusters and identities. Together, our results demonstrate that hMOs can be used to investigate ubiquitin-based interventions to modulate PCD sensitivities. Pati-

ent-derived hMOs will facilitate research in personalized therapies bridging in vitro research and clinical outcome.

0079 STAT3 isoforms – Striking the balance

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The signal transducer and activator of transcription 3 (STAT3) plays a vital role in cell proliferation, differentiation, and apoptosis, and – not surprisingly – its constitutive activation is associated with a bad prognosis in cancer. Two alternatively spliced isoforms of STAT3 exist, – full-length STAT3α and the truncated isoform STAT3β. These two isoforms seem to play opposite roles in acute myeloid leukemia (AML), and we have shown previously that a higher STAT3β/α ratio in AML blasts is associated with prolonged survival. Here, we studied the potential of different drugs to modulate this ratio. We identified two drugs, atovaquone, and selinexor, that both have the capability to increase the STAT3β/α ratio. The combined treatment of atovaquone and selinexor showed even synergistic killing of leukemia cells and further elevation of the STAT3β/α ratio. The higher ratio led to an upregulation of the STAT3β target gene CD62L and reduced tumor cell migration in vitro. The combination of atovaquone and selinexor resulted in additional elevation of CD62L expression and CD62L overexpression in THP-1 cells, significantly prolonged survival in a xenograft model.

0080 Dissecting enhancers at nucleotide resolution in pediatric cancer

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Genome-wide association studies revealed that most of disease-associated variants map at non-coding regions. Mechanistically, some of these single nucleotide variants have been shown to alter transcription factor binding affinity, leading to aberrant transcriptional regulation and thereby leading to disease. In this context, we aim at dissecting enhancers carrying germline variants predisposing to pB-ALL. To setup the required technology, we have studied two loci relevant to immunotherapy in pediatric cancer, PD-L1 and CD19. We screened a large regulatory region upstream of PD-L1 in neuroblastoma cells using CRISPRi, and identified a 2.5kb fragment, retaining most of the regulatory activity and enriched in TEAD binding motifs. Using base editors, we dissected a CD19 enhancer at nucleotide resolution in a leukemia cell line. By comparing allele frequencies in CD19 high and low cells, we identified MYB, PAX5 and IKZF1 binding motifs, alongside nucleotide substitutions that disrupt TF binding resulting in diminished CD19 levels. These alleles might be relevant in the context of resistance to aCD19 CAR T cell therapy.

0081 Investigating the Role of CTCF in Antisense Transcription: Insights and Implications in AML

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Our recent findings indicate that CTCF, a versatile transcription factor, can block transcription of non-coding upstream antisense RNAs without influencing sense coding genes at bidirectional promoters. CTCF binding pattern changes occur frequently in cancer and can result from DNA methylation alterations or mutations in the CTCF itself. Therefore, we hypothesize that altered CTCF occupancy in acute myeloid leukemia (AML) can lead to aberrant antisense transcription, hence contributing to oncogenesis. In order to identify CTCF binding loci that impact upstream antisense transcription, we will utilize the mini-Auxin-inducible degron system to rapidly degrade CTCF in leukemia cell lines following auxin treatment. We are also investigating antisense expression in pediatric AML patients and healthy donors using RNA-seq. Cancer-specific antisense RNAs will be tested for functionality using CRISPR screens. This study aims to uncover cancer vulnerabilities at the level of CTCF-dependent gene regulatory elements and non-coding RNAs, which could be exploited as biomarkers of disease or as therapeutic targets.

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