Abstract Book

XXXII. Jahrestagung der Kind-Philipp-Stiftung
für Leukämieforschung
in Wilsede
vom 5. – 7. Juni 2019

UKH Universitätsklinikum Halle (Saale)

Medizinische Fakultät der Martin-Luther-Universität Halle-Wittenberg

MHH Medizinische Hochschule Hannover

Princes MÁXIMA centrum voor kinderoncologie

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

Ausrichter (organized by):
Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Halle; Institut für Pharmazeutische Biologie, Goethe Universität Frankfurt am Main; Pädiatrische Hämatologie und Onkologie, Medizinische Hochschule Hannover; Princess Maxima Center for Pediatric Oncology, Utrecht

PROGRAM AT A GLANCE

Wednesday, June 5	Thursday, June 6	Friday, June 7
	9:00-10:45 Molecular mechanisms of disease I	9:00-10:30 Immunotherapy
	11:00-12:45 Drugs I	10:45-12:30 Molecular mechanisms of disease III
	12:45-14:15 Lunch at Heidemuseum	12:30-13:45 Lunch at Heidemuseum
	14:15-15:45 Diagnostics and Biomarkers	13:45-15:00 Molecular mechanisms of disease IV
16:00-17:30 Welcome and Registration	16:15-17:45 Molecular mechanisms of disease II	15:15-16:45 Drugs II
17:30-18:30 Invited Lecture: T. Mercher	18:15-19:00 Invited Lecture: M. Suttorp	16:45-17:15 Wilsede Award, Farewell
18:30-19:45 Omics Studies	19:00 Barbecue	17:15 coaches to Undeloh 18:00 Bus transfer to HH-Dammtor
19:45 Dinner		

WEDNESDAY, June 5, 2019

(2) Omics studies

Chair: Thomas Sternsdorf

2.a Mutational and transcriptomic landscapes in hematopoietic cells of Fanconi anemia patients.

Mittapalli VR[1], Andrieux G[2], Hochrein J[2], Vukicevic V[1], Kratz CP[3], Niemeyer CM[1], Börries M[2], Erlacher M[1]

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Fanconi anemia (FA) is a rare genetic disease caused by deficient DNA damage repair. Patients are at risk to develop bone marrow failure (BMF) and secondary myelodysplastic syndrome (MDS) and MDS-related acute myeloid leukemia (MDR-AML). In this project, we aim at understanding the mechanisms of hematopoietic failure and identifying driver mutations and dysregulated pathways involved in progression to MDS/AML. For this, we collected bone marrow samples from FA patients suffering from the different disease stages (i.e. BMF, low-grade MDS, advanced MDS and AML) and subjected them to WES and RNAseq. Preliminary transcriptome data revealed downregulation of DNA repair pathways and upregulation of pro-apoptotic genes in BMF samples. During disease progression towards MDS/AML, mutations in oncogenes and tumor suppressors accumulated. Our work will contribute to a greater understanding of the role of mutations and their deregulated gene expression in the advancement of FA-associated MDR-AML.

2.b Mapping the single cell transcriptome reveals the cellular composition of ATRT subgroups

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Atypical Teratoid/ Rhabdoid Tumors (ATRTs) are paediatric brain cancers, occurring mostly in infants. The driving mutation is a biallelic loss of the SMARCB1 gene, a member of the SWI/SNF complex. Epigenetic studies revealed three distinct molecular subgroups of ATRTs (TYR, SHH, MYC) associated with specific clinical and (epi)genomic features. To survey the cellular composition of the three ATRT subgroups, we performed single nucleus RNA-sequencing of 25.000 cells from 12 ATRT samples (TYR: 3, SHH: 6, MYC: 3). Overall tSNE clustering analyses of the RNA data revealed distinct tumor and non-tumor cell populations (such as microglia), the latter most abundantly present in MYC and SHH. Moreover, we detected one cluster of cells, almost exclusively present in TYR that exhibited the transcriptomic profile of neuronal stem cells. On the contrary, SHH harboured many tumor cells that displayed an OPC like differentiation. In summary, single cell transcriptome analyses revealed communalities and differences in the cellular composition of ATRTs, revealing different stem cell-like cell populations in each ATRT subgroup and suggesting that each subgroup may have a different cell of origin.

2.c Giant cell glioblastoma does not represent a distinct entity but stratifies into different genetically defined entities

Wiedey A[1], Doerner E[1], zur Muehlen A[1], Kramm C[2], Waha A[1], Pietsch T[1]

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Giant cell glioblastoma (G-GBM) is a rare variant of glioblastoma characterized by very large often multinucleated tumour cells. G-GBM occur most frequently de novo; in contrast to IDH wildtype GBM they carry TP53 mutations in high frequency. We performed a systematic (immuno)histological and genetic analysis by MIP, panel NGS and pyrosequencing in a cohort of 58 G-GBM (25 children, 33 adults). 78.4% of G-GBM carried mutations of TP53. Five G-GBM represented IDH-mutated gliomas, 5 cases had H3F3A-G34, and 3 had H3-K27M mutations. The vast majority (75%) lacked IDH or histone gene mutations. In these, chromosome 7 showed a copy number gain in 2/3 of the cases. TERT promotor mutations occurred only in adult patients at a frequency of 35.5%. Chrom. 1q gain, 10 and 13 loss was more frequent in adults. MGMT promotor was methylated in 30.6 % of G-GBM. As a potential therapeutic target, PDGFR-alpha was found expressed in most cases and its gene showed copy number gain in 34.5%. In summary, G-GBM is a histological phenotype most likely related to p53

dysfunction but can occur in different genetically defined GBM entities (IDH-, histone gene mutated or in IDH/histone wildtype GBM).

2.d WNT Medulloblastoma: More than just β-Catenin Mutations – Rare Genetic Features in the Focus

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WNT-activated medulloblastomas (MB) account for ~10% of all MB cases and represent low-risk tumours with overall survival rates >90% in children. Nearly all WNT MB show classic histology and widespread nuclear accumulation of β-catenin related to CTNNB1 mutations (>90%). In CTNNB1 wildtype tumours APC mutations have been described. Additional TP53 mutations (in ~15% of cases) have been published as not correlated to worse outcome. Apart from monosomy 6 (~85%), WNT tumours have relatively stable genomes. In a cohort of 152 WNT MB we identified APC mutations in 9 of 14 CTNNB1-wildtype tumours with chr. 5q aberrations in 8 of these 9 cases by molecular inversion probe array, NGS, and Sanger sequencing. In the other 5 wildtype cases one AXIN2 mutation and one homozygous loss of FBXW7 led to WNT activation, while in 3 cases no causative mutations were found. In 9 relapsed WNT tumours we identified 4 TP53 mutations; in one case a de novo mutation was found in the relapse. TP53 mutations were associated with chr. 17p loss and p53 nuclear positivity. The clinical relevance of such alternative/additional mutational events has to be analyzed in homogenously treated patient cohorts.

2.e Epigenetics profiling for minimal residual disease in paediatric acute myeloid leukaemia

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Next-generation sequencing (NGS) has been used for adult acute myeloid leukaemia (AML) diagnosis with some success using the Illumina TruSight Myeloid Sequencing Panel (Illumina) which detects mutations in 54 genes commonly found in patients with hematologic cancers. Unfortunately, paediatric AML has one of the lowest rates of mutation among molecularly well characterized cancers, with less than 1 somatic, protein-coding change per mega base in most cases, leading to many false negatives with the above described method. Therefore, there is a need to develop more widely applicable molecular or genomic-based assay for paediatric AML. Aberrant DNA methylation patterns are a characteristic feature of AML. Several studies have evaluated AML genome-wide methylation. Each of these DNA methylation-defined AML subtypes displayed a unique epigenetic signature when compared with the normal bone marrow CD34+ cells. Epimutations, unlike classical gene mutations, are not due to changes in DNA sequence but in the methylation signatures. However, just like normal mutations these epimutations can be used in MRD diagnosis for treatment and stratification purposes. Furthermore, epimutation profiling

THURSDAY, June 6, 2019

(3) Molecular mechanisms of disease I

Chair: Owen Williams

3.a Splicing factors of the SF3b complex PHF5A and SF3B1 regulate the DNA damage response in ALL

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To identify potential regulators of propagation and self-renewal of Acute Lymphoblastic Leukaemia (ALL), we performed a genome-wide RNAi screen that identified the splicing factor PHF5A. PHF5A is a subunit of the SF3b protein complex together with SF3B1, which has also been implicated in several haematological malignancies. We show that PHF5A and SF3B1 knockdown significantly reduces cell proliferation and colony forming ability. Moreover, knockdown of PHF5A and SF3B1 induce DNA strand breaks as indicated by comet assay and increased γ-H2AX levels. Using RNA-seq datasets, we validated candidate genes which have been affected by differential splicing and/or differential expression. Notably, PHF5A and SF3B1 knockdown reduced FANCD2 expression and induced skipping of exon 22 associated with impaired mono-ubiquitination of the FANCD2 protein. Furthermore, expression of RAD51, a key DNA repair factor, also decreased upon PHF5A and SF3B1 knockdown. Taken together, our data show that splicing factors regulate the DNA Damage response in ALL.

3.b Inactivation of Nsd1 impairs terminal erythroid maturation and induces erythroleukemia

Tauchmann S[1], Almosailleakh M[1], Leonards K[1], Otzen Bagger F[1], Juge S[1], Thirant C[2], Méreau H[1], Peters AHFM[3], Mercher T[2]; Schwaller J[1]

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Nuclear interacting SET domain protein 1 (NSD1) is fused to NUP98 in pediatric AML, however its role in hematopoiesis is unknown. We found that loss of NSD1 altered clonogenic growth of cord blood-derived CD34+ cells leading to accumulation of erythroid progenitors. Ablation of Nsd1 during murine fetal liver hematopoiesis led to a fully penetrant lethal erythroleukemia-like disease. In vitro terminal erythroid maturation of Nsd1-/- erythroblasts was significantly impaired and associated with constitutive expression of the erythroid master regulator GATA1. Retroviral expression of Nsd1, but not a catalytic-inactive mutant rescued the terminal erythroid differentiation of Nsd1-/- erythroblasts associated with upregulation of erythroid regulators on mRNA and protein level. Differentiation of Nsd1-/- erythroblasts was not only associated with increased expression of proposed GATA1 target genes and accompanied by increased GATA1 chromatin binding, but also with reduced interaction to potent transcriptional repressors. Collectively, we identified NSD1 as a regulator of terminal erythroid maturation by controlling the transactivation potential of the erythroid master regulator GATA1.

3.c TRIM28 haploinsufficiency predisposes to Wilms tumor

Metzler M[1], Diets IJ[2], Hoyer J[3], Wegert J[4], Graf N[5], Vokuhl C[6], Gessler M[4], Kuiper RP[7], Jongmans MCJ[8]

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Background: Most Wilms tumor diagnoses occur in sporadic patients. About 2% of cases have one or more relatives affected by Wilms tumor. The currently known repertoire of oncogenic driver mutations includes WT1, CTNNB1, AMER1, MYCN, SIX1/2 and several miRNA processing genes. The underlying genetic cause of some

familial cases remains unexplained, indicating the existence of other Wilms tumor predisposition genes. **Method**: Two families with two affected individuals were analyzed using germline exome sequencing. Additional 269 children affected with Wilms tumor were screened for mutations in this gene by targeted enrichment sequencing. **Results**: We identified heterozygous germline truncated mutations in TRIM28 in eleven children that become homozygous in the Wilms tumor tissue. The tumors showed an epithelial-type histology that stained negative for TRIM28 by immunohistochemistry. MRNA and protein level of TRIM28 were reduced suggesting that loss of TRIM28 is the main driver of tumorigenesis. **Conclusions**: Our data identify TRIM28 as a novel Wilms tumor predisposition gene, functioning as a classical tumor suppressor gene in Wilms tumor.

3.d CRISPR-mediated genome editing of t(4;11) in human prenatal and perinatal hematopoietic stem/ progenitor cells

Torres-Ruiz R[1], Bueno C[1], Velasco-Hernandez T[1], Roca-Ho H[1], Gutierrez-Aguera F[1], Quintana O[2], Segovia JC[2], Roy A[3], Rodriguez-Perales S[4], Menendez P[1]

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Rearrangements of the MLL gene are associated with aggressive acute leukemia. The most common MLL-rearrangement is MLL-AF4 which is the hallmark genetic abnormality of infant t(4;11) pro-B ALL, it also has one of the lowest mutation rates among cancers. It is associated with poor prognosis and displays a very brief latency, raising the question of how this disease evolves so rapidly. Despite worldwide efforts, progress about its aetiology, pathogenesis and cellular origin remains unresolved. In order to contribute to these unresolved questions, we have applied a CRISPR/Cas9-mediated genome editing strategy to recreate the t(4;11) translocation in HSPC isolated from different ontogeny stages including fetal liver and cord blood derived HSPCs. The genome edited HSPCs at distinct developmental stages have been functionally assayed to address whether t(4;11) initiates leukemogenesis on its own or whether secondary cooperating hits are required for an overt leukemia. These pioneering studies will reveal a precise understanding on the impact of such chromosomal rearrangements on leukemogenesis, and provide a humanized disease model, offering a platform for new treatment strategies.

3.e Therapeutic application of the tumour suppressive miR-193b in acute myeloid leukaemia

Issa H, Bhayadia R, Klusmann JH

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Dysregulation of microRNAs (miRNAs) is a hallmark of leukaemogenises. Many miRNAs regulate leukaemic cell proliferation by modulating several kinases or interfering with the expression of key proto-oncogenes. miR-193b expression is globally downregualted in Acute Myeloid Leukaemia (AML) and we found that low endogenous level of miR-193b is an independent marker for poor prognosis in adult and paediatric AML. Restoring the expression of miR-193b might provide a therapeutic window. Ectopic lentiviral expression of miR-193b or transient induction of miR-193b mimics reduced CCND1 expression, induced G1 cell cycle arrest and triggered apoptosis in various AML cell lines and patient blasts in vitro. The regulatory mechanism of miR-193b was found to be driven by targeting several components of MAPK pathway including the KIT/RAS/RAF/MEK/ERK cascade. We are developing a lipid nanoparticle based delivery system to restore the tumour suppressor activity of miR-193b in AML patient derived xenografts in vivo and exploiting this therapeutic intervention with current AML treatment.

3.f Genome-wide CRISPR screen in patient derived cells reveals the mechanism of ALL chemoresistance

Szoltysek K[1], Dormon K[1], Blair H[1], Tirtakusuma R[1], Dodd C[1], Law E[1], Nakjang S[1], Pal D[1], Vormoor J[2], Heidenreich O[1]

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ALL (acute lymphoblastic leukaemia) treatment is associated with significant infection-related mortality that comes as a consequence of currently used genotoxic drugs. Despite of therapy improvement, occurrence of cancer relapse results in a relatively poor outcome and still remains one of the major therapeutic challenges. Here, we performed a study to investigate the mechanism of dexamethasone resistance in a model of TCF3-HLF (t(17;19)-positive) ALL. We have performed a genome-wide CRISPR screen in patient-derived xenografts (PDX)

both in vitro and in vivo. For the in vitro approach we have used co-culture system of patient-derived material with different types of human stromal cells (mesenchymal and endothelial-like), which allowed us to recreate oncogenic microenvironment as well as to provide support for growth and proliferation of primary ALL samples in vitro. We have identified the glucocorticoid receptor NR3C1 as the gene which deletion possibly drives the mechanism of chemoresistance in this type of high risk ALL. Most importantly, we have shown that genome-wide screens are able to predict the relapse in primary ALL samples.

3.g CRISPRi screen to identify functional long noncoding RNAs in pediatric acute myeloid leukemia

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Long noncoding RNAs (lncRNAs) have emerged as new regulators of gene expression. Large-scale studies highlight their precise expression patterns, and many are implicated in pathophysiological processes, but the vast majority still lack functional characterization. We previously profiled lncRNA expression in the human blood system and in pediatric acute myeloid leukemia (AML) samples, and found stem cell and subtype-specific signatures in AML blasts. To identify functional candidates, we screened 619 of these lncRNAs in a CRISPRi-based dropout approach. Of 26 hits, 10 were validated via proliferation assays and qPCR. One candidate stood out as essential in 6 of the 8 cell lines tested – LNC666, a nuclear-enriched transcript whose knockdown is marked by megakaryocytic M-07es. It is flanked by two coding genes, both of which seem dispensable for AML cells as shown by CRISPR-Cas9 knockout. Meanwhile, shRNAs against LNC666 and gene excision both led to a growth disadvantage, with similar results in patient-derived xenografts in vivo. In healthy CD34+ cells, LNC666 knockdown reduced erythroid differentiation. Our results suggest LNC666 as an important lncRNA for the pathogenesis of AML.

(4) Drugs I

Chair: Torsten Pietsch

4.a Tumour Necrosis Factor receptor (TNFR)-signalling dependent killing in T-cell acute lymphoblastic leukaemia (T-ALL)

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Autocrine TNF can promote cell survival and leukaemia progression and is associated with poor prognosis. SMAC-mimetics (SM), pharmacological inhibitors of IAPs, are critical regulators of TNF-signalling and are able to sensitise leukaemias to TNF-induced cell death. Here, we examine the mechanism of SM-sensitivity by investigating individual components of the TNF-system. We found constitutive low level expression of TNFR1, whereas cell surface TNFR2 was detected at various expression levels in approximately 30% of T-ALLs. As expected, SM birinapant-induced cell death was dependent on TNF production and promotes TNF-induced cell death in various T-ALL patient-derived xenografts (PDX). Interestingly, correlation analyses revealed a very good correlation between SM-sensitivity and cell surface TNFR2. In line with this, shRNA-mediated knockdown of TNFR2 is sufficient to significantly diminish TNF/SM-induced cell death in T-ALL cell lines, whereas ectopic expression of TNFR2 primed TNFR2-negative T-ALL cell lines for TNF/SM-induced cell death. Together, our data demonstrate heterogeneous expression of TNFR2 in T-ALL, which determines the killing capacity of Smac-mimetic birinapant.

4.b iPSC Model of Stepwise Leukemia Development in Congenital Neutropenia Reveals BAALC as a Key Mediator of Leukemogenesis

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Recently, we reported a high frequency of cooperating RUNX1 and CSF3R mutations in CN patients that developed AML or MDS. We established a model for step-wise leukemia progression in CN using iPSC-based hematopoietic differentiation in combination with CRISPR/Cas9-mediated gene editing of iPSCs. Using this model, we confirmed that co-acquisition of CSF3R and RUNX1 mutations is sufficient to induce leukemia in CN. In addition, we identified BAALC (brain and acute leukemia, cytoplasmic) upregulation as a key leukemogenic event downstream of RUNX1 and CSF3R mutations. BAALC mRNA was upregulated in primary CN/AML blasts (n=5) and in CD34+ HSPCs generated from CN/AML iPSCs of two patients. Importantly, CRISPR/Cas9-mediated knockout of BAALC in CN/AML-iPSCs inhibited proliferation and induced myeloid differentiation of CN/AML blasts. Using connectivity Map analysis of RNA-Seq data of iPSC-derived CD34+ cells, we identified a p38 MAPK/MK2 inhibitor that could possibly reverse BAALC-mediated leukemogenic gene expression signature. Proliferation of primary CN/AML blasts, CN/AML-iPSC-derived CD34+ cells and BAALC-expressing de novo AML blasts was decreased upon treatment with this inhibitor.

4.c Targeting c-MYB in Acute Leukaemia through Drug Repositioning

Clesham KJ, Gasparoli L, Virely C, Cantilena S, De Boer J, Williams O

UCL Great Ormond Street Institute of Child Health, UCL, London, UK.

Novel treatments are urgently needed for childhood acute myeloid leukaemia (AML). The transcription factor c-MYB plays a central role in the development and maintenance of AML. We hypothesised that deregulation of c-MYB could be a therapeutic approach. We identified Withferin A (WfA) as a candidate for such therapy using connectivity MAP analysis. WfA induced degradation of c-MYB in AML cell lines, and demonstrated anti-leukaemic properties in vitro, inducing apoptosis and increased expression of differentiation antigens, and inhibiting colony formation. We provide initial evidence that a major part of WFA activity is through inhibition of oncogenic activation of c-MYB target genes: enrichment of a c-MYB activated gene list in drug-induced gene expression changes; partial rescue of colony formation and protein degradation in response to transient exposure to WFA, following expression of degradation-resistant c-MYB in AML cells. This work will now be

extended to explore the mechanism by which WFA is modulating c-MYB, and its efficacy in primary patient-derived cells in vitro and in vivo.

4.d Functional analysis of class I HDAC inhibition in group 3 medulloblastoma to identify synergistic drug combinations

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Medulloblastoma (MB) is a highly aggressive childhood brain tumour. Patients with Group 3 MB harbouring a MYC-amplification show particularly poor outcome. We and others have previously shown that MYC-amplified MBs are highly susceptible towards class I histone deacetylase inhibition (HDACi). In clinical trials HDACi monotherapy shows only modest efficacy in solid tumours. We here delineate the molecular effects of class I HDACi in MB to identify potentially synergistic drug combinations. The MYC-amplified MB cell line HD-MB03 was treated with class I HDACi entinostat. Transcriptional changes were determined by gene expression profiling, qPCR and western blot and functionally assessed using Gene Set Enrichment Analysis, Ingenuity Pathway Analysis and Cytoscape. Entinostat treatment evokes up/downregulation of various pharmacologically targetable biomechanisms including MHC-antigen processing, vesicle processing, DNA damage, RNA processing, cell cycle, p53 and kinase signalling. We hypothesize a synergistic effect of entinostat and agents targeting these biomechanisms. In vitro synergy studies in MYC-amplified vs non MYC-amplified MB cell lines are currently ongoing.

4.e Screening assay to identify potential Taspase1 inhibitors

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Introduction: Taspase1 is necessary to process the MLL protein and the oncogenic AF4-MLL fusions protein. In order to activate Taspase1, two proenzymes have to form a homodimer which causes an autoprotolytical activation. Unfortunately, Taspase1 is no classical "enzyme" rather than a "single action protein". Methods: A special HTRF-assay has been designed in combination with special Taspase1 mutants as read-out system to monitor Taspase1 activity over longer time periods (up to 48h). In addition, we also established a cell-free system to measure Taspase1 activity. Results & Conclusion: The developed screening assays allow to perform high-throughput screens to identify potential inhibitors for the treatment of t(4;11) leukemia patients.

4.f Analyzing the therapeutic efficacy of navitoclax and MCL-1 inhibitors in juvenile myelomonyctic leukemia

Wu Y, Koleci N, Gallego-Villar L, Mittapalli VR, Bohler S, Erlacher M

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Juvenile myelomonocytic leukemia (JMML) is a highly aggressive myeloid malignancy of early childhood, caused by constitutive RAS pathway activation and epigenetic deregulation. Most patients require hematopoietic stem cell transplantation but recently, the DNA methyltransferase inhibitor azacitidine was shown to have unprecedented activity against JMML, both in patients and in our xenograft model. Here we aim at investigating whether so-called BH3-mimetics can be used to treat JMML. For this, we are treating JMML xenograft mice with either ABT737 (a navitoclax analogue) or S63845, two BH3-mimetics inhibiting BCL-2/BCL-XL or MCL-1, respectively. In addition, we are analyzing whether low-dose azacitidine can sensitize JMML towards these drugs. While high-dose azacitidine showed efficient depletion of leukemic cells and, most importantly, leukemia-initiating cells (LICs), treatment with ABT737 resulted in a reduction of leukemic infiltrations but not LICs. In vitro, MCL-1 inhibition showed stronger cytotoxic activities on patient-derived JMML cells than BCL-2/BCL-XL inhibition.

4.g Establishment of new therapeutic options for the treatment of Myeloid Leukemia in Down syndrome (ML-DS)

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Children with DS have a 400-fold increased risk of developing myeloid leukemia (ML-DS). Although ML-DS has a good prognosis, children with DS particularly suffer from the side effects of polychemotherapy. Hence, there is an urgent need for the development of new, more sustainable therapeutic agents. Expression studies and ML-DS modeling in mice revealed high expression of antiapoptotic BCL2-family member BCL-XL as well as derepression of Lysine-specific demethylase 1A (LSD1) target genes. Therefore, BCL inhibitor ABT-263 (Navitoclax) and LSD1 inhibitors (iLSD1) present as promising drug candidates against ML-DS. Various AML cell lines as well as leukemic blasts form AML patients were subjected to serially diluted concentrations of ABT-263 and iLSD1 in vitro, CD34+ cells served as control. Of note, ML-DS leukemic blasts and cell lines exhibited clear antiproliferative response with IC50 values of 362 nM and 33 nM, whereas for CD34+ cells the IC50 was not achieved. The effectiveness of Navitoclax and iLSD1 highlight the importance of the corresponding pathways in the maintenance and progression of ML-DS and give an outlook towards unexplored targeted therapeutic opportunities.

(5) Diagnostics and Biomarkers

Chair: Roland Kappler

5.a Masked hypodiploidy against uniparental disomies in hyperdiploid ALL: Observations from the diagnostics perspective

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Optimal treatment of pediatric B-cell precursor acute lymphoblastic leukemia (pB-ALL) relies on individual risk stratification of patients. Hypodiploidy represent a rare high risk group and is challenging to detect in many cases due to doubling of hypodiploid karyotypes (masked hypodiploidy), thereby creating numerous whole chromosome uniparental disomies (UPDs). For this reason, cases of pB-ALL were subjected to CGH+SNP microarray analysis (n=447), which allows for detection of numerical and structural alterations including masked hypodiploidy and the recently discovered risk marker IKZF1plus. Masked hypodiploidy was discovered in seven cases. Notably, two masked hypodiploidies presented numerous trisomies alongside expected UPDs and tetrasomies. This indicates a more complex clonal evolution of masked hypodiploidy for some cases as appreciated in the literature to date. Interestingly, hyperdiploid karyotypes presented UPD of one or more chromosomes in about 20% of cases, demanding for a longitudinal analysis on whether these losses of heterozygosity might influence leukemogenesis and prognosis.

5.b Large amplicon droplet digital PCR for DNA-based monitoring of pediatric chronic myeloid leukemia (CML)

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Background: In the era of tyrosine-kinase inhibitor treatment stop trials in patients with CML, quantification of BCR-ABL1 fusion is an essential tool to guide treatment decisions. For detection of transcript-negative CML cells, DNA-based assays are an alternative approach. However, repeat-rich DNA sequences at the breakpoint limit the design of conventional PCR assays for minimal residual disease (MRD) monitoring. **Method**: Droplet digital PCR (ddPCR) was optimized for quantification of large amplicons (<1330 bp) to allow primer/probe set design outside repeat regions. DNA and RNA BCR-ABL1 copy numbers of 687 specimens from 55 pediatric CML patients were compared. **Results**: Molecular characterization of genomic BCR-ABL1 fusion sites from pediatric CML patients revealed repeat elements at the breakpoint in 64% of cases. Using ddPCR, primers can be positioned outside repeat regions and large amplicons can be quantified with high sensitivity. **Conclusions**: The combination of ddPCR, double quenched probes and extended amplicons represents a valuable tool for MRD monitoring in CML and may be adapted to other translocation positive tumors.

5.c Stratification and prognosis of IGH-DUX4 positive acute lymphoblastic leukemia in children

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Introduction: Recently B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with the presence of an IGH-DUX4 rearrangement was identified as a novel genetic subtype. The aim of the study presented here was to describe the prognostic relevance of IGH-DUX4 in patients treated according to the AIEOP-BFM ALL 2009 protocol. **Methods**: Patients diagnosed between 2010 and 2014 and negative for known recurrent genetic abnormalities (B-others) were included. We used extracted DNA of leukemic bone marrow and peripheral blood cells and performed two different Multiplex-Long Range PCR-assays to detect all IGH-DUX4 rearrangements described in the literature so far. Demultiplexed PCR assays and Sanger sequencing were used to verify positive results. **Results**: 396 BCP-ALL cases were analyzed. In 29 of the 396 samples the IGH-DUX4 rearrangement was detected and verified (7.3%). Only 1 of the 29 patients with IGH-DUX4 positive ALL was stratified as standard risk patient, 16 as medium risk and 12 as high risk patients. Four of the 29 patients relapsed. **Conclusion**: The IGH-DUX4 Rearrangement constitutes a genetic intermediate risk profile.

5.d Multiplex droplet digital PCR-based targeted enrichment NGS for identification of tumor markers in Ewing sarcoma (EwS)

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Background: Genomic EWSR1-FLI1 fusion sequences have been used as non-invasive tumor marker to assess therapy response in EwS patients by quantification of cell-free circulating tumor DNA in plasma samples. Since fresh-frozen tumor biopsies are often not available, fusion sequences have to be identified in highly fragmented DNA from formalin-fixed, paraffin-embedded (FFPE) specimen. Methods: Patient-specific EWS fusion genes were pre-amplified in a multiplex droplet digital PCR (mddPCR) using DNA from FFPE tumor samples. This library was then sequenced with the Ion S5 System. Results: The combination of mddPCR and targeted enrichment NGS improves the success rate for identification of genomic fusion sequences and facilitates their use as a biomarker for ctDNA monitoring. Analyses of ctDNA levels and clinical parameters from EWING2008 patients revealed correlation of ctDNA copy numbers and patients' risk factors. Discussion: MddPCR based targeted enrichment NGS strategy enables the identification of tumor specific fusion sequences from highly fragmented DNA samples and therefore allows their application as molecular tumor marker in a wider range of sample sources.

5.e Machine learning algorithms for the automated classification of pediatric anemia

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Anemia is a common laboratory finding in children, caused by a multitude of common to rare and minor to life-threatening diseases. Current diagnostic tests enable early and accurate recognition of the underlying diagnosis, but their use varies with physician experience and patient setting. We investigated whether machine learning algorithms for the automated interpretation of pediatric blood counts can support the differential diagnosis of anemia. A comprehensive database of pediatric blood counts (13,913 healthy children and 1,668 pathological samples: 29.3% iron deficiency anemia, 9.2% Blackfan Diamond Anemia, 39.9% myelodysplastic syndrome, 16.4% vitamin B12-deficiency, and 5.2% heterozygous β-Thalassemia) was split into training and validation datasets to develop different machine learning models. The best performing models predicted the diagnoses in the validation dataset with an accuracy of 99.9 % (classification as either "normal" or "abnormal") and 93.9 % (classification as "healthy" or specific diagnoses). This provides a proof-of-concept application of machine learning algorithms to support the diagnosis of complex hematological diseases in children.

5.f Identification of the genetic mechanisms linked to the occurrence of H3.3K27M mutation in pediatric diffuse intrinsic pontine gliomas

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Pediatric high-grade gliomas (pedHGGs) account for 10-15% of pediatric CNS tumors with very poor prognosis. 30-40% of pedHGG carry a mutation in lysine 27 of histone 3 (H3K27M) resulting in an even worse prognosis. Although it is known that H3K27M-mutation leads to global loss of H3K27 trimethylation (H3K27me3) with hyperacetylation of H3K27, the underlying biological mechanism is unknown. We hypothesize that the H3K27M-induced hyperacetylation (H3K27ac) by histone acetyltransferases, such as CREB Binding Protein (CBP), might be accompanied by a greater impact of acetylation-dependent epigenetic readers, such as Bromodomain and Extra Terminal domain proteins (BET). To identify the probable distinct epigenetic and resulting tumor-biological effects caused by H3K27M-mutation within the same cellular and genetic background, isogenic cell lines with H3K27M-mutation and with H3 wildtype will be compared with each other. Additionally, to investigate the role of CBP and BET proteins in dependence on the H3-mutation status, siRNA-mediated knockdown will be performed. In summary, this project aims to determine the underlying mechanisms induced by H3K27M-mutation to identify therapeutic targets for affected patients with pedHGG.

(6) Molecular mechanisms of disease II

Chair: Deepali Pal

6.a Deciphering the interactive network of the DLK1-DIO3 locus in hematopoiesis and pediatric acute megakaryoblastic leukemia

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Non-coding RNAs (ncRNAs) recently emerged as central regulators of chromatin and gene expression, posing a novel window for targeted therapies in pediatric acute megakaryoblastic leukemia (AMKL). We established a lncRNA expression atlas for the hematopoietic system including 46 pediatric acute myeloid leukemia samples, and discovered a ncRNA cluster within the DLK1-DIO3 locus that was highly expressed in hematopoietic stem cells (HSCs), megakaryocytes (MKs) and AMKL. ChIP-Seq performed for human CD34+ hematopoietic stem and progenitor cells (HSPCs), MKs and monocytes showed cell type specific activating (H3K3me3) and repressing (H3K27me3) histone marks. Bisulphite sequencing revealed a significant correlation between MEG3 expression and the methylation status of a CpG island downstream of the first exon of MEG3. Lentiviral expression of several highly expressed miRNAs of the cluster in CD34+ HSPCs resulted in accelerated MK maturation in vitro. CRISPR-Cas9-mediated deletion of MEG3 impaired proliferation of AMKL cell lines. Our study establishes the DLK1-DIO3 locus as an important regulator of megakaryopoiesis with different members controlling this process.

6.b Identification of GATA1s interaction partners in Down syndrome-associated myeloid leukemia

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Children with Down syndrome (DS) are at high risk of developing myeloid leukemia (ML-DS). Up to 30% of newborns with DS develop a pre-leukemic transient abnormal myelopoiesis (TAM). TAM is characterized by GATA1 mutations (GATA1s) that result in a shorter form lacking the N-terminal transactivation domain. How trisomy 21 cooperates with GATA1s in TAM development is not fully understood. In a CRISPR/Cas9 screen, RUNX1 lost resulted in depletion of ML-DS cells. Additionally, we observed differential RUNX1 isoform expression in AMKL (non-DS) and ML-DS primary cells compared to normal hematopoietic stem/ progenitor cells or terminally differentiated cells. In a newly established TAM/ML-DS assay, GATA1s synergized with particular isoforms leading to a hyperproliferative phenotype in vitro and induction of leukemia in vivo. This was further confirmed by co-immunoprecipitation assays followed by mass spectrometric analysis and DNA sequencing, showing differences in the physical interactions of GATA1/GATA1s and RUNX1 isoforms as well as at genomic loci in TAM and ML-DS. These results highlight the importance of analyzing all isoforms of a gene when studying its function in leukemogenesis.

6.c Isolation and characterization of tumor-derived exosomes from a patient-derived xenograft mouse model of acute leukemia

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Exosomal cargo has been found dysregulated in various neoplasias, including acute leukemias. Yet, in primary patients' material, it is uncertain whether the found biomarkers are derived from tumor exosomes (TEX) or from surrounding tissue. To circumvent this problem, we use a patient-derived xenograft (PDX) mouse model of acute leukemia. We capture serum exosomes with a human specific antibody against the exosomal marker CD63. Exosomes and their RNA are analyzed with human specific cytometry and PCR assays to confirm the human origin. Our assay reveals high sensitivity, detecting human exosomes in human:mouse serum titrations down to a ratio of 1:100 and in the serum of PDX mice. Bioanalyzer analysis confirms a distinctive enrichment of small RNAs. Exosome levels and their RNA amounts are increased in full-blown leukemia mice compared to control mice. Our human-specific exosome capture assay indicates that leukemic cells are utilizing exosomes to communicate with their microenvironment, and potentially distant tissues. Applying this method, we will sequence TEX-derived RNA from different ALL risk groups to detect high risk patients upfront with a serum sample at time of diagnosis.

6.d Interrogating the role of chromatin regulator BRD4 in the DNA damage response in medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor in children. MYC-driven tumors make up 40% of MBs and display the poorest prognosis. Treatment often has debilitating effects on patients, thus, novel molecularly targeted therapies could minimize adverse effects and improve treatment. Bromodomain and Extra-terminal domain (BET) inhibitors were introduced as an alternative strategy for targeting MYC proteins, by impairing epigenetic regulation of MYC transcription. Emerging data show that MYC repression is not the only effect that BET inhibition has on various cancers. We interrogated the role of chromatin regulator BRD4 in the DNA damage response in MB. We showed that small-molecule BET inhibition can down-regulate key DNA repair proteins of various DNA repair pathways, and that BET inhibitors and BET degraders synergize with other established chemotherapeutic compounds in MB cell lines. We further demonstrated that transcriptional repression results in defective DNA repair, suggesting that the development of drug-combination therapies including BET-inhibitors and established first-line MB agents may enhance drug efficacy in MB patients.

6.e Analyzing the effects of oncogenic SHP2 on apoptosis signaling during malignant transformation

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Juvenile myelomonocytic leukemia (JMML) is a very aggressive myeloproliferative neoplasia of early childhood. The most aggressive subtype is caused by mutations in PTPN11 coding for SHP2 that lead to activation of the RAS/MAPK, PI3K/AKT and Rho signaling pathways. Apoptosis deregulation is an important hallmark in leukemia and it is known that apoptosis-resistant cells are selected during malignant transformation. We have a special interest in the BCL-2 protein family that consists of pro- and anti-apoptotic members. It is known that RAS activation increases cell survival by regulating BCL-2 proteins but it is unclear whether and how BCL-2 proteins are involved in the different stages of RAS driven transformation. To address this, we use PTPN11 knockin mice prone to myeloproliferation and leukemia to investigate the regulation and roles of anti- and pro-apoptotic BCL-2 proteins before, during and upon malignant transformation. We are analyzing BCL-2 protein regulation and apoptotic susceptibility in differentiated myeloid and monocytic cells as well as in stem and progenitor cells, and testing BH3-mimetics for their therapeutic potential in patients with JMML.

6.f Chromosome structure and mitotic defects are major pathogenic mechanisms in hyperdiploid childhood B-ALL.

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High-hyperdiploid B-cell acute lymphoblastic leukemia (HHD-ALL) is defined by the presence of 51-67 chromosomes. HHD is thought to be the initiating oncogenic event in this subtype of B-ALL but the molecular mechanisms leading to HHD remain unknown. The aim of this work was to study the contribution of mitotic defects to the origin of HHD-ALL. We set out to study the contribution of mitotic defects in BCP-ALL primary samples. Results showed that HHD-ALL cells grow significantly slower than Non-HHD-ALL. Immunofluorescence analysis showed an accumulation of HHD-ALL cells in prometaphase coupled with defects on chromosome biorientation and increased chromosome missegregation rates. HHD-ALL cells showed high-order chromosome architecture defects with reduced SMC2 levels at chromosome scaffolds and Aurora B kinase misslocalization from the centromere. Aurora B defects were coupled with cohesion defects and an impaired spindle assembly checkpoint, leading to mitotic slippage and increased apoptotic rates. Results suggest that chromosome condensation defects associated with Aurora B misslocalization in prometaphase is a major pathogenic mechanism contributing to the origin of HHD-ALL.

FRIDAY, June 7, 2019

(8) Immunotherapy

Chair: Olaf Heidenreich

8.a Development of a novel central nervous system (CNS)-targeted CD19-antibody (AB) in acute lymphoblastic leukemia (ALL)

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Introduction: The limited ability of drugs to cross the blood-brain barrier (BBB) hampers the eradication of leukemic blasts from the CNS. Furthermore, a relative lack of effector cells in the CNS can preclude the use of AB-based immunotherapies in CNS leukemia. We aimed to create a CD19-AB able to cross the BBB and exert ALL-specific cytotoxicity in the CNS. Method: A CD19-AB was fused to the BBB shuttle peptide Angiopep2 (An2) and cytotoxic tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Binding and killing of the AB were determined in vitro. For in vivo imaging, fluorescently labeled CD19-AB was injected into immunodeficient mice bearing CD19+ ALL cells. Results: We engineered a CD19-An2-TRAIL-AB showing specific binding to CD19+ ALL cells as well as U87 MG glioma cells carrying the An2-mediated transcytosis promoting receptor LRP1. CD19-An2-TRAIL-AB induced programmed cell death in CD19+ ALL cells. Using IRDye coupling, we established an in vivo imaging technique to visualize binding of CD19-ABs to ALL cells in the CNS.

8.b CD19 and CD22-directed bispecific CAR for B-cell acute lymphoblastic leukemia

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CD19 CAR-T cells have shown impressive rates of clinical response in B-cell acute lymphoblastic leukemia (B-ALL) but CD19- relapses are still common. A combinational targeting of multiple antigens represents a potential strategy to overcome this, so we developed a CAR containing binding domains for CD19 and CD22 in tandem (CD22/CD19 CAR) and we cloned it in a pCCL lentivector. Lentiviral particles were produced and used to transduce primary pre-activated human T cells (anti CD3/CD28 plus IL7 and IL15) and we tested pre-clinically the activity of our CD22/CD19 CAR for B-ALL. Using CRISPR/Cas9-edited CD19+CD22+, CD19+CD22-, CD19-CD22+ and double KO SEM cells we confirmed in in vitro cytotoxic assays the bispecificity of the CD22/CD19 CAR. In vitro and in vivo cytotoxic assays revealed that CD22/CD19 CAR achieves very similar cytotoxic potency than CD19 CAR but with significantly lower in vitro production of the pro-inflammatory cytokines IL-2, INF-γ and TNF-α which may have major implications in lowering the rates of cytokine release syndrome toxicity. Further experimental work is underway to assess whether the CD22/CD19 CAR can contribute to delay/prevent long-term relapses.

8.c Low regulatory T-cells are associated with improved survival of neuroblastoma patients treated with anti-GD2 antibodies

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Immunotherapies with anti-GD2 antibodies (Abs) have improved survival of high-risk neuroblastoma (NB) patients (pts). In a closed single-center program, 53 pts received 5 cycles of 6×106 IU/m2 subcutaneous IL-2 (d1-

5; 8-12) combined with long-term infusion (LTI) of 100 mg/m2 of the anti-GD2 Ab ch14.18/CHO (d8-18). Cytotoxic NK-, regulatory T cells (Tregs) and neutrophils were analyzed by flow cytometry. IFN-γ, IL-6, IL-10, IL-18 and CCL2 serum concentrations were measured using bead-based immunoassays. All data were correlated with PFS. IL-2 administration increased cytotoxic NK- and Treg cell counts in cycle 1 followed by further increase in subsequent cycles, whereas neutrophil levels were elevated only after the Ab infusion. Levels of IFN-γ, IL-6, IL-10, IL-18 and CCL2 were increased after the combined therapy. Importantly, pts with low Treg levels had significantly improved PFS compared to pts with high levels. Notably, Treg counts negatively correlated with INF-γ concentrations. In conclusion, LTI of ch14.18/CHO in combination with IL-2 resulted in Treg induction that negatively correlated with IFN-γ release and PFS.

8.d CRISPR/Cas9 Gene-modification Platform of ELANE Mutations in iPSCs and HSPCs of Severe Congenital Neutropenia Patients

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Severe congenital neutropenia (CN) is a monogenic bone marrow failure syndrome characterized by an absolute neutrophil count below 500/ul. Autosomal-dominant ELANE mutations are the common cause of CN. ca. 15 % do not respond to G-CSF therapy at doses up to 50 µg/kg/day and ca. 15% of G-CSF treated patients developed MDS or AML. We aimed to develop a platform for CRISPR/Cas9 ribonucleoprotein (RNP)- or AAV- mediated gene correction or knockout of ELANE in induced pluripotent stem cells (iPSC) and primary HSPCs of CN patients. We observed that granulocytic differentiation of ELANE KO iPSC and HSPCs was comparable to healthy individuals and phagocytic function of the ELANE KO neutrophils also was normal. Simultaneously, CRISPR/Cas9 mediated correction of more severe ELANE mutations enables neutrophilic maturation of gene edited HSPCs and iPSCs of CN patients. Moreover, using whole transcriptome analysis, we identified key signaling pathways regulated by mutated ELANE in CN HSPCs. In summary, we established CRISPR/Cas9 based gene-modification platform for CN patient`s HSPCs that can be also applied for the treatment of patients with other monogenic bone marrow failure Syndromes.

8.e Venetoclax enhances the efficacy of therapeutic antibodies in B-cell malignancies

Heymann J[1], Vogiatzi F[1], Rösner T[2], Lenk L[1], Cario G[1], Schrappe M[1], Valerius T[2], Peipp M[2], Kellner C[2], Schewe DM[1]

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Aim: In B-cell-neoplasias, patients with high Bcl-2 expression often have a poor prognosis. This includes patients with double-hit lymphomas (DHL), Burkitt´s lymphoma (BL) and t[17;19]-positive ALL. We examined the role of the Bcl-2 inhibitor Venetoclax (VTX) on the efficacy of therapeutic antibodies. Methods: Antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated phagocytosis (ADCP) were measured in cell line and PDX models of DHL, BL and t[17;19]-ALL with the antibodies Rituximab (RTX), Daratumumab (Dara) and CD19-DE (a proprietary engineered antibody) in combination with VTX. Effects of VTX on the ADCP capacities of Rituximab (RTX) and CD19-DE were examined in vivo in xenograft mice. Results: ADCC and CDC were not enhanced by VTX. Increased ADCP by human and murine macrophages were detected in DHL cell lines with VTX/RTX and VTX/Dara, in BL-PDX with VTX/RTX and in t[17;19]-PDX with VTX/CD19-DE. Mice treated with VTX/RTX and VTX/CD19-DE showed superior survival as compared to VTX, RTX or CD19-DE alone. Conclusion: VTX enhances the efficacy of antibodies in B-cell-malignancies in vitro and in vivo.

8.f Blockade of suppressive myeloid cells is effective against neuroblastoma

von Lojewski, L

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Neuroblastoma (NB) is a challenging pediatric cancer with a 5-year survival rate below 50% in high-risk patients. Improvements were achieved with anti-GD2 therapies, but many patients still relapse. CD11b+ immune suppressive cells of myeloid lineage represent a promising target, as their immunosuppressive role in tumorigenesis was shown. Here, we analyzed myeloid-derived suppressor cells (MDSC) and the expression of MDSC-associated genes in a syngeneic NB mouse model. To show tumor promoting effects of CD11b+ cells and MDSC, these two cell populations were blocked by anti-CD11b monoclonal antibody administration or selectively depleted by low dose 5-Fluorouracil (5-FU) treatment, respectively. High numbers of CD11b+ leukocytes and a strong tumor-dependent induction of MDSC-associated genes could be shown by flow cytometry, immunohistochemical and RT-PCR analyses. MDSC depletion by both anti-CD11b and 5-FU treatment (superior effect) reduced the expression of MDSC-associated genes, delayed tumor growth and improved survival, suggesting a negative role of MDSC in NB. In conclusion, the depletion of immune suppressive myeloid cells resembles a promising treatment strategy against NB.

(9) Molecular mechanisms of disease III

Chair: Jasper de Boer

9.a Too little is too much: Leukemogenesis in the Gata2 syndrome

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GATA2 is a transcription factor required for the generation/maintenance of hematopoietic stem cells. Strikingly, up to 75% of GATA2 haploinsufficient individuals develop myelodysplastic syndromes with a high risk of progression to leukemia. As the mechanism behind it is unknown, we aimed at deciphering how GATA2 haploinsufficiency drives leukemogenesis. We hypothesized that GATA2 haploinsufficiency either directly induces leukemia or provokes bone marrow failure, which later develops into secondary leukemia. To test these hypotheses, we analyzed the oncogenic potential of Gata2+/- in proliferation assays and after mutagen treatment. In parallel we assessed their susceptibility to provoke bone marrow failure by subjecting them to different cell stresses (ageing, transplantations). We discovered that Gata2+/- cells produced premalignant cells in vitro and accelerated leukemogenesis in vivo. Transplantation of Gata2+/- cells resulted in bone marrow failure. Our results show that Gata2 haploinsufficiency drives on the one hand leukemogenesis and on the other hand fuels bone marrow failure. We are now investigating the link and the mechanism behind this intriguing paradoxal behavior.

9.b Using acute promyelocytic leukemia to study the H3.3 histone chaperone system and its role in pediatric malignancies

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Cancer genome projects have discovered frequent mutations in genes for Chromatin-remodeling-factors in pediatric tumors. These include genes encoding Daxx and ATRX, two proteins involved in deposition of histone variant H3.3, indicating a role for H3.3 deposition in carcinogenesis. The macromolecular complex harboring Daxx/ATRX is also the target of the Acute Promyelocytic Leukemia oncoprotein PML-RARα. PML-RARα leads to a disruption of the localization of Daxx/ATRX, physiologically found at PML nuclear bodies, which can be reversed by treatment with All-Trans-Retinoic Acid (ATRA). We used this to search for novel components of the PML-associated Daxx/ATRX complex using Proximity-mediated Biotin Identification (BioID). We have used a BioID-Daxx bait protein to identify proteins interacting with Daxx in APL cells in an ATRA-dependent manner by Mass Spectrometry. Using this method, we have identified the Swi/Snf-ATPase subunit SMARCA4 as a protein interacting with Daxx.. This provides an unexpected link between two seemingly distinct Chromatin-modulating mechanisms, H3.3 incorporation (Daxx/ATRX) and nucleosome remodeling (Swi/Snf), which might open up novel therapeutic possibilities

9.c Functional characterization of RUNX1 variants in the context of FPDMM

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Germline variants in RUNX1 cause familial platelet disorder with associated myeloid malignancies (FPDMM). The functional impact of RUNX1 missense variants is often unclear, so further investigations are needed to determine their clinical relevance. RUNX1 binds CBFβ and together they function as a transcriptional regulator. To functionally characterize 9 variants of unknown significance (VUS), we performed western blotting, FACS-FRET assay, and luciferase reporter assays with 4 independent reporters in HEK293T cells. As a proof of principle, 6 known pathogenic RUNX1 variants were investigated in parallel. In comparison with RUNX1 wild type, we showed significant functional impairment for all known pathogenic variants and gained evidence for the functional relevance of 6 RUNX1 VUS. They showed reduced ability to activate transcription of different reporters. Impaired dimerization capacity to CBFβ and/or reduced RUNX1 phosphorylation was detectable.

Additionally, these RUNX1 VUS accumulated in the cells indicating altered degradation. Our results help to elucidate the clinical impact of RUNX1 VUS. In the future, additional assays will be developed to characterize VUS in more detail.

9.d FOS and FOSB are linked with CNS-infiltration and inferior prognosis in childhood T-cell acute lymphoblastic leukemia

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Aim: CNS involvement in ALL is a major clinical challenge, particularly in T-ALL. Novel targets to reliably detect and eradicate CNS-leukemia are urgently needed. Methods: Comparative RNA-sequencing was performed with patient derived xenograft TALL blasts recovered from the bone marrow (BM) and the CNS of NSG-mice. FOS and FOSB mRNA were measured in diagnostic bone marrow samples of 112 pediatric T-ALL patients and correlated with clinical parameters. Results: The AP-1 genes FOS and FOSB were significantly upregulated in blasts recovered from the CNS versus BM of NSG-mice. Accordingly, CNS+ patients exposed significantly elevated FOSB-mRNA levels as compared to CNS- patients (p=0.038). Furthermore, FOShigh and FOSBhigh patients (mRNA levels above median) showed significantly lower 5-year event free survival (p=0.031 and p=0.011, respectively) than FOSlow and FOSBlow patients. Importantly, 6/9 patients with CNS-relapse were FOShigh and FOSBhigh upon diagnosis. Conclusion: FOS and FOSB may be novel independent predictors of prognosis and surrogate markers with therapeutic potential for CNS-infiltration and relapse in T-ALL requiring further prospective and mechanistic validation.

9.e Deciphering role of lncRNAs in pediatric AML

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Acute myeloid leukemia (AML) is a hematopoietic malignancy defined by genetic (and epigenetic) alterations in hematopoietic stem or progenitor cells. The emerging picture portrays a chromatin and transcriptional landscape that precisely controlled in four dimensions (space, time, cell type, developmental status) by a range of regulatory long non-coding RNAs (lncRNAs). Previously, we generated a gene expression atlas uncovering prognostically relevant lncRNA signatures shared between various AML sub-types and healthy hematopoietic stem cells. To elucidate the relevance of lncRNA candidates in leukemogenesis we performed an in vivo CRISPRi-based dropout screen. We targeted leukemic cell lines with various mutational sub-types and patient derived xenografts with stable expression of dCas9-KRAB. In our in vivo experimental design, firstly, we determined frequency of leukemia-initiating cells using a barcode approach. Thereafter we subjected our lncRNA candidate sub-libraries for screening potential candidates. Dropout candidates of the screening are further being validated via proliferation assays and qPCR.

9.f Genomic characterisation of lineage switched MLL-rearranged leukemias

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Unlike other MLL rearrangements, t(4;11) is strongly associated with ALL. Here we have characterised the lineage switch from ALL to AML which carry identical MLL breakpoint. Nine t(4;11) and one t(9;11) cases were defined by loss of B lymphoid and gain of myeloid antigens and/or unequivocal changed to myeloid morphology. Performed RNAseq and DNase-seq analyses revealed myeloid-like transcriptional reprogramming in relapse samples, as well as chromatin reorganisation associated with changed TFs occupation of lymphoid and myeloid genes on presentation and relapse, respectively. The fusion MLL/AF4 gene can be detected in the multipotent

progenitor (MPP) cells in both presentation and relapse, indicating an early HSPC as a potential cell of origin. Notably, mutations in PHF3 and CHD4, correlated with epigenetics regulation, were also identified in the relapse MPP. Moreover, knockdown of these genes in SEM cells and CD34+ chimeric MLL/Af4 cells trigger a myeloid programme. Taken together, these data show that the strong lymphoid potential of MLL/AF4 depends on the CHD4 and PHF3, and that mutations in these epigenetic regulators direct the switch towards the myeloid lineage.

9.g Downstream effect of CSF3R and RUNX1 mutations that underlie leukemic transformations in congenital neutropenia (CN)

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Ca. 70% of CN patients with AML carry CSF3R and RUNX1 mutations (Skokowa et al 2014). We established an in vitro model utilizing lin- bone marrow cells from C57BL/6-1d715csf3r mice (with homozygous d715G CSF3R mutations) transduced with lentivirus vectors carrying RUNX1 wildtype or RUNX1 missense mutations. Cells transduced with RUNX1 mutants showed diminished in vitro myeloid differentiation and elevated replating capacity, compared to WT. mRNA expression analysis of transduced cells using Pathway analysis (IPA) and Motif activity response analysis (using ISMARA) revealed that the highest activated motif in RUNX1-Mutants was Irf2_Irf1_Irf8_Irf9_Irf7 motif. Correspondingly, IPA Pathway analysis showed that Interferon Signaling was highly upregulated in cells transduced with RUNX1 mutants, compared to WT RUNX1. Additionally, pathway analysis showed the upregulation and activation of IL-6, IL-8-, Toll like Receptor- and TREM1 signaling pathways. This data suggests that the mutated RUNX1 may cause activation of the pro-inflammatory cell state propagating proliferation, which may be emerging as a cause of clonal hematopoiesis and consequently may lead to MDS/AML.

(10) Molecular mechanisms of disease IV

Chair: Markus Metzler

10.a Identification of a new splice variant of the human transcription factor ONECUT2

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Using a cDNA library from the chemo-resistant Hodgkin lymphoma (HL) cell line L-1236, we identified a new splicing variant of the human transcription factor ONECUT2 (OC2). This new variant (OC2s) contains a single CUT domain without an associated HOX domain, which characterizes the proteins of the ONECUT family. Expression analysis by quantitative Real Time PCR (qRT-PCR) showed a high expression of OC2 and OC2s in HL cell lines as well as in normal liver tissue. In contrast, the cell lines Kasumi-1, HL-60 and MCF-7 as well as normal testis showed high expression of OC2s only. The majority of normal tissues and cell lines expressed only low levels of OC2 and OC2s. We observed that knockdown of OC2 lead to decreased OC2s expression whereas transgenic over-expression of OC2 had no impact on OC2s. Interestingly, over-expressed OC2 was located predominantly in the cytoplasm and not, as expected, in the nucleus. Gene expression analysis of cells with altered expression of OC2 or OC2s revealed further candidates regulated by these factors.

10.b NAMPT-mediated LMO2 deacetylation is indispensable for hematopoiesis and T-ALL leukemogenesis

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LMO2 (hematopoietic transcription factor LIM domain only 2), a member of the TAL1 transcriptional complex, plays an essential role during early hematopoiesis and is frequently activated in T cell acute lymphoblastic leukemia (T-ALL) patients. Here, we demonstrated that LMO2 is activated by deacetylation on lysine 74 and 78 via the nicotinamide phosphoribosyltransferase (NAMPT)/sirtuin 2 (SIRT2) pathway. LMO2 deacetylation enables LMO2 to interact with LDB1 and activate the TAL1 complex. NAMPT/SIRT2-mediated deacetylation of LMO2 is essential for hematopoietic differentiation of induced pluripotent stem (iPS) cells and blood formation in zebrafish embryos. In T-ALL, deacetylated LMO2 induces expression of TAL1 complex target genes HHEX, NKX3.1 as well as LMO2 autoregulation. Consistent with this, inhibition of NAMPT or SIRT2 suppressed the in vitro growth and in vivo engraftment of T-ALL cells via diminished LMO2 deacetylation. This new molecular mechanism may provide new therapeutic possibilities in T-ALL and may contribute to the development of new methods for in vitro generation of blood cells.

10.c GADD45b plays an essential role in the G-CSF triggered granulocytic differentiation of human hematopoietic cells

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The mechanism of maturation arrest of granulopoiesis in congenital neutropenia (CN) is not fully elucidated. We found that GADD45b is induced by G-CSF in healthy HSPCs, but not in CN cells. We inhibited GADD45b expression in CD34+ cells and iPSCs by CRISPR/Cas9 RNP and found markedly diminished granulocytic differentiation in GADD45b knockout iPSCs and CD34+ cells. Transduction of CN patient HSPCs with GADD45B cDNA restored granulocytic differentiation. In silico analysis of GADD45B promoter, reporter gene and ChIP assays revealed CEBPA-mediated activation of GADD45b. GADD45b is regulating active DNA demethylation by promoting the recruiting of DNA demethylation machineries to specific genomic loci. Therefore, we investigated whether G-CSF-triggered granulocytic differentiation of HSPCs requires regulation of active gene demethylation by GADD45b using RNA sequencing and the Infinium methylation EPIC array of WT or GADD45b-edited CD34+ HSPCs treated or not with G-CSF. G-CSF treatment of HSPCs resulted in the robust changes of DNA methylation that was markedly reduced in GADD45b KO cells. We identified several signaling pathways that are regulated by GADD45b-mediated demethylation.

10.d Tripartite motif-containing 71 (TRIM71) is a major factor of oncogenic activity in human hepatoblastoma

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Introduction: Hepatoblastoma (HB) is the most common type of liver tumor in children and generally associated with a good prognosis, if not presenting as a multifocal, vessel invasive or metastatic tumor. Therefore, a better understanding of the molecular mechanisms driving aggressive growth of HB is necessary. Methods: Gene expression was determined by real-time PCR and RNA sequencing. Gain and loss of function experiments were conducted in 4 liver tumor cell lines to assess proliferation, apoptosis, mobility, and self renewal. Results: An initial RNA sequencing approach identified TRIM71 as a candidate gene in HB. Expression analysis of a comprehensive patient cohort validated TRIM71 to be significantly upregulated in HB. Overexpression of TRIM71 in low expressing cells led to a significantly reduced proliferation rate and increased self renewal capacity, while leaving apoptosis and mobility unchanged. Conversely, TRIM71 knockdown in high expressing cells led to an increased growth rate and reduced self renewal. Transcriptomic analyses highlight the importance of stemness genes in these biological processes. Conclusion: TRIM71 plays a major role in the aggressiveness of HB.

10.e Cooperativity between miR-125b and Gata1s in the pathogenesis of Down syndrome-associated myeloid leukemia

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Down syndrome-associated myeloid leukemia is characterized by the triad of trisomy 21, fetal origin and mutations in GATA1 (GATA1s mutations). However, the synergy of trisomy 21 and GATA1s in disease initiation and progression remains to be understood. Leveraging combined CRISPR-Cas9 genome editing and lentiviral overexpression, we interrogated the interaction of the Gata1s mutation with different permutations of members of the miR-99a~125b tricistron (miR-125b-2, miR-99a, let-7c) in murine fetal liver cells. We observed major synergistic effects by the combination of Gata1s with miR-125b, leading to increased proliferation of immature progenitor cells in vitro and induction of leukemia in both primary and secondary recipients. To obtain molecular insights, an shRNA screening individually probing miR-125b targets, combined with RNA-Seq analysis upon inducible miR-125b expression, uncovered the transcription factor Arid3a as the main miR-125b target involved in this process. In conclusion, we present miR-125b cooperates with Gata1s – in the fetal context – in enhancing proliferation and transformation of hematopoietic progenitors.

(11) Drugs II

Chair: Julia Skokowa

11.a Investigating HDACi and dnTaspase1 for the treatment of (4;11) leukemic cells

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Introduction: Class I HDACi has been shown to be effective for t(4;11) fusion proteins, as it abolishes functions from MLL-AF4 (to set a leukemic expression profile) while wildtype MLL becomes activated. In addition, functions deriving from AF4-MLL (to set active chromatin) can be counteracted by a dnTapsase1 mutant (dnTASP1), which in turn causes the degradation of the AF4-MLL fusion protein. Here we evaluated the combination of both treament options on viability and apoptosis of t(4,11) cells. Methods: IC50 values were elucidated for three cass I HDACi drugs and used for a specific treatment schedule that allows to measure viability and apoptosis in transgenic SEM cells, expressing dnTASP1 in a Doxycyclin-inducible fashion. Results: & Conclusion: Class I HDACi are potent inhibitors that can be still enhanced by the expression of dnTASP1. Data of this optimized protocol in conjunction with standard chemotherapeutics on the survival will be presented.

11.b Dasatinib and dexamethasone offer a novel therapeutic strategy for T-cell acute lymphoblastic leukaemia

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T-cell Acute Lymphoblastic Leukaemia (T-ALL) is caused by malignant transformation of T cells showing differentiation arrest and uncontrolled proliferation. The checkpoints during T-cell development are dominated by pre-T-cell receptor (pTCR) for β-selection and T-cell receptor (TCR) for positive/negative selection. LCK is a central molecule in pTCR/TCR signalling transduction. To investigate the importance of pTCR/TCR complex for T-ALL cell proliferation and survival, a targeted in vitro and in vivo shRNA screen in 4 cell lines and 2 PDXs identified LCK to be crucial for T-ALL maintenance and engraftment. Mechanistic analyses indicate that knockdown or inhibition of LCK by Dasatinib impairs cell proliferation by inducing G1/G0 arrest. Moreover, LCK knockdown significantly sensitises cells to Dexamethasone (Dex), and strong synergistic lethal effects between Dex and Dasatinib have been observed in various cell lines and PDXs. A randomised phase II-like trial in NSG mice demonstrates a significant reduction in leukaemia burden after combination treatment. The Dex/Dasatinib combination might provide a novel treatment strategy for refractory and relapsed T-ALL patients.

11.c Drug repositioning in infant leukaemia

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Introduction: Despite an overall improvement in the prognosis of childhood leukaemia, survival rates for infants still remain poor. Many of these leukaemias are driven by mixed lineage leukaemia (MLL) gene rearrangements. Therefore, a therapy that could degrade MLL-rearranged genes could be a potential treatment. Drug repositioning, identifying a novel clinical use for an already approved drug, is a more beneficial route than typical drug discovery as it uses de-risked compounds developed with lower costs over a shorter time. Previously the lab carried out a drug screen to identify clinically approved drugs that could degrade MLL fusion proteins. The purpose of this study was to validate and characterise one of these positive hits. Methods: Western blot was used to measure MLL fusion protein degradation and qPCR was used to measure gene expression. TOPRO assays were used to measure cell death. Results: We showed in a panel of human MLL-rearranged leukaemic cell lines that drug treatment reduced expression of MLL fusion protein and its target genes and affects cell death. Conclusion: We have validated this drug as a positive hit and continue to characterise its action.

11.d Synthetic lethality of Wnt pathway activation and asparaginase in drug-resistant acute leukemias

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Resistance to asparaginase is a common clinical problem, whose biologic basis is poorly understood. We hypothesized, from the concept of synthetic lethality, that gain-of-fitness alterations in drug-resistant cells had conferred a survival advantage that could be exploited therapeutically. Using a genome-wide CRISPR/Cas9 screen in T-ALL, we found that negative regulators of Wnt signaling were selectively depleted upon treatment with asparaginase. Wnt pathway activation induced profound asparaginase sensitivity in distinct treatment-resistant subtypes of acute leukemia, but not in normal hematopoietic progenitors. Sensitization to asparaginase was mediated by Wnt-dependent stabilization of proteins (Wnt/STOP), which inhibits GSK3-dependent protein ubiquitination and proteasomal degradation, a catabolic source of asparagine. Inhibiting the alpha isoform of GSK3 was sufficient to phenocopy synthetic lethality with asparaginase, and pharmacologic GSK3 alpha inhibition profoundly sensitized drug-resistant leukemias to asparaginase in vitro and in vivo. These results demonstrate that synthetic lethal drug-drug interactions can improve the therapeutic index of cancer therapy.

11.e A human BM-iPSC-derived oncogenic niche identifies CDH2 as therapeutic niche target in leukemia

Pal, D; *Newcastle upon Tyne*

NO ABSTRACT AVAILABLE

11.f Inhibition of the polycomb repressive complex 1 (PRC1) as a therapeutic option in childhood liver tumors

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Introduction: Hepatoblastoma (HB) is the most common childhood liver tumor. A component of the repressive PRC1 complex has been found to be mutated in some HBs indicating a role in HB biology. **Methods**: After measuring expression levels of different PRC1 components in HB samples via qPCR, we tested the effects of pharmacological PRC1 inhibition on cell proliferation and clonogenic growth of HB cell lines. We performed apoptosis and senescence assays. Expression of possible PRC1 target genes was measured by RNA sequencing. **Results**: We found that the PRC1 component BMI1 is significatly upregulated in HB and higher levels are correlated with metastasis, multifocality and a worse outcome. Pharmacological inhibtion resulted in decreased proliferation and clonogenic growth and increased apoptosis. The BMI1 inhibitor showed strong synergistic effects with cisplatin. **Conclusion**: BMI1 is a promising target for pharmacological inhibition in HB.