and Obstetrics, University Hospital of Geneva, Geneva, Switzerland. [13]CANSEARCH research platform for Pediatric Oncology and Hematology, Faculty of Medicine, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland. [14]Department of Oncology, University Children's Hospital, Zurich, Switzerland. [15]Division of Pediatric Hematology/Oncology, Department of Pediatrics and Adolescent Medicine, Medical University of Graz, Graz, Austria. [16]Clinical Cooperation Unit Neuropathology (B^{300}), German Cancer Research Center (DKFZ), and German Cancer Consortium (DKTK), Heidelberg, Germany. [17]Department of Neuropathology, Heidelberg University Hospital, Heidelberg, Germany. [18]Institute for Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

PURPOSE/METHODS: Clinical and molecular risk factors in 142 patients <5 years with desmoplastic medulloblastoma (DMB) or medulloblastoma with extensive nodularity (MBEN) were investigated. Patients were diagnosed between 1992 and 2020 and treated with radiation-sparing approaches, 131 with intraventricular methotrexate. 14 patients with metastatic disease received high-dose chemotherapy. DNA methylation profiles of 77 sonic hedgehog (SHH)-activated medulloblastoma were reclassified according to the Heidelberg Brain Tumor Classifier Version 12.3. RESULTS: While metastatic disease or incomplete resection did not impact progression-free survival (PFS) and overall survival (OS), patients with MBEN had superior outcomes to DMB (5-year PFS 93% vs 71%, p=0.004; 5-year OS 100% vs 90%, p=0.026). Older patients had less favorable PFS (5-year PFS [>3 years] 47% vs 85% [<1 year] vs 84% [1-3 years], p<0.001). No TP53 mutations were detected (n=47). DNA methylation classification identified three subgroups: SHH-$1_{v12.3}$ (n=39), SHH-$2_{v12.3}$ (n=19), and SHH-$3_{v12.3}$ (n=19), with distinct cytogenetic profiles (chromosome 2 gains in SHH-$1_{v12.3}$, very few alterations in SHH-$2_{v12.3}$, and chromosome 9q losses in SHH-$3_{v12.3}$), age profiles (median age [years] SHH-$1_{v12.3}$: 1.7, SHH-$2_{v12.3}$: 0.9, SHH-$3_{v12.3}$: 3.0, p<0.001), and histological distribution (SHH-$2_{v12.3}$: 74% MBEN, SHH-$1_{v12.3}$/SHH-$3_{v12.3}$: 77%/79% DMB, p<0.001). PFS was more unfavorable in patients with SHH-$3_{v12.3}$-medulloblastoma (5-year PFS 53% vs 86% [SHH-$1_{v12.3}$] vs 95% [SHH-$2_{v12.3}$], p=0.002), which remained the only risk factor on multivariable Cox regression for PFS. OS was comparable (5-year OS 94% [SHH-$3_{v12.3}$] vs 97% [SHH-$1_{v12.3}$] vs 100% [SHH-$2_{v12.3}$], p=0.6). 8/9 patients with SHH-$3_{v12.3}$-medulloblastoma received radiotherapy at relapse (6 craniospinal, 2 local [1 Gorlin syndrome, 1 BRCA2 germline mutation], 1 no radiotherapy [Gorlin syndrome]). CONCLUSION: We identify patients with an increased risk of relapse when treated with radiation-sparing approaches among children with early childhood SHH-medulloblastoma. If these tumors differ from SHH-3-medulloblastoma typically described in older children remains to be verified. Treatment recommendations need to consider cancer predisposition syndromes.

MEDB-42. GERMLINE *ELP1* DEFICIENCY PROMOTES GENOMIC INSTABILITY AND SURVIVAL OF GRANULE NEURON PROGENITORS PRIMED FOR SHH MEDULLOBLASTOMA PATHOGENESIS

Jesus Garcia-Lopez *[1], Shiekh Tanveer Ahmad *[1], Yiran Li *[1], Brian Gudenas[1], Marija Kojic[2], Friedrik Manz[3], Barbara Jonchere[1], Anand Mayasundari[1], Aaron Pitre[1], Jennifer Hadley[1], Leena Paul[1], Melissa Batts[1], Brandon Bianski[1], Christopher Tinkle[1], Brent Orr[1], Zoran Rankovic[1], Giles Robinson[1], Martine Roussel[1], Brandon Wainwright[2], Lena Kutscher[3], Hong Lin #[1], Paul Northcott #[1]; [1]St. Jude Children's Research Hospital, Memphis, TN, USA. [2]Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia. [3]Division of Pediatric Neuro-oncology, Heidelberg, Germany

Germline loss-of-function (LOF) mutations in *Elongator complex protein 1 (ELP1)* are found in 15-20% of childhood SHH medulloblastoma (MB) and are exceedingly rare in non-SHH-MB or other cancers. *ELP1* germline carriers that develop SHH-MB harbor frequent somatic *PTCH1* mutations and universally sustain loss-of-heterozygosity of the remaining *ELP1* allele through chromosome 9q deletion. ELP1 functions as a scaffolding subunit of the Elongator complex that is required for posttranscriptional modification of tRNAs and maintenance of efficient translational elongation and protein homeostasis. However, the molecular, biochemical, and cellular mechanisms by which ELP1/Elongator LOF contribute to SHH-MB tumorigenesis remain largely unknown. Herein, we report that mice harboring germline *Elp1* monoallelic loss (i.e., *Elp1*+/-) exhibit hallmark features of malignant predisposition in developing cerebellar granule neuron progenitors (GNPs), the lineage-of-origin for SHH-MB. *Elp1*+/- GNPs are characterized by increased replication stress-induced DNA damage, upregulation of the homologous recombination repair pathway, aberrant cell cycle, and attenuation of p53-dependent apoptosis. CRISPR/Cas9-mediated *Elp1* and *Ptch1* gene targeting in mouse GNPs reproduces highly penetrant SHH-MB tumors recapitulating the molecular and phenotypic features of patient tumors. Reactivation of the p53 pathway through MDM2 and PAK4 inhibitors promotes selective cell death in patient-derived xenograft tumors (PDX) harboring deleterious *ELP1* mutations. Together, our findings reveal that germline *Elp1* deficiency heightens genomic instability and survival in GNPs, providing a mechanistic model for the subgroup-restricted pattern of predisposition and malignancy associated with pathogenic *ELP1* germline carriers. These results provide rationale for further preclinical studies evaluating drugs that overcome p53 pathway inhibition in *ELP1*-associated SHH-MB and a renewed outlook for improving treatment options for affected children and their families.*, # Contributed equally

MEDB-43. DEVELOPMENT OF A BIOINFORMATICS PIPELINE FOR IDENTIFICATION OF DIFFERENTIAL DNA METHYLATION EVENTS ASSOCIATED WITH MEDULLOBLASTOMA RELAPSE

Christopher Kui[1], Stacey Richardson[1], Edward C Schwalbe[1,2], Dean Thompson[1,2], Claire Keeling[1], Gordon Strathdee[3], Christelle Dufour[4], Simon Bailey[1,5], Vijay Ramaswamy[6], Steven C Clifford[1], Rebecca M Hill[1,5]; [1]Wolfson Childhood Cancer Research Centre, Translational and Clinical Research Institute, Newcastle University Centre for Cancer, Newcastle University, Newcastle-upon-Tyne, United Kingdom. [2]Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne, United Kingdom. [3]Biosciences Institute, Newcastle University Centre for Cancer, Newcastle University, Newcastle-upon-Tyne, United Kingdom. [4]Department of Pediatric and Adolescent Oncology, Gustave Roussy, [94800] Villejuif, France. [5]Great North Children's Hospital, Newcastle-upon-Tyne Hospitals NHS Foundation Trust, Newcastle-upon-Tyne, United Kingdom. [6]The Hospital for Sick Children, Toronto, Ontario, Canada

Relapsed medulloblastoma (rMB) is treatment-resistant and fatal in ~95% of cases. The epigenetic features of rMB, and any role as drivers of disease relapse/treatment-resistance have yet to be investigated. We therefore developed a pipeline to identify differentially methylated CpGs (DM-CpGs) and regions (DMRs) in a paired-rMB cohort. Our paired-rMB cohort (n=61, relapsed tumours matched with diagnosis counterparts) with available Illumina Methylation 450K/850K microarray data was processed in *R-Studio*. The packages *Limma* and *DMRcate* were used to perform a paired differential methylation analysis on a filtered selection of array probes (n=335,767), identifying DM-CpGs and DMRs with a 5% FDR. DMRs were further retained if they had a maximum-$\Delta\beta$ of >0.2 and correlated with locus-specific gene expression in a separate paired DNA-methylation array/RNA-seq cohort from medulloblastoma diagnosis samples (n=202). Finally, we created univariable Cox models to assess the prognostic potential of DM-CpGs/DMRs in an independent survival cohort of medulloblastoma diagnosis samples (n=498). Across the paired-rMB cohort, there were few significant differential methylation events initially identified at relapse (n=258 DM-CpGs, n=32 DMRs). Upon sub-analysis by molecular group, MB_{Group4} (n=18 pairs) alone yielded significant findings (n=189 DM-CpGs, n=26 DMRs). Most changes involved hypermethylation events detected at relapse. Multiple DM-CpGs identified at relapse were prognostic for both overall and event-free survival when assessed in our independent cohort (n=22 whole cohort, n=13 Group 4, BH-adjusted p<0.05). When applying the DMR filters, only the MB_{Group4} DMRs passed the $\Delta\beta$ filter (n=18/26), with few correlating with gene expression (n=2, p<0.001), and none demonstrating prognostic significance. This pipeline facilitates exploration of the clinical relevance of epigenome-wide changes in a paired-rMB cohort. We highlight the potential prognostic significance of DM-CpGs, and future work will explore the potential functional role of candidate-genes associated with our DMRs, as novel drivers of rMB.

MEDB-44. TRANSCRIPTOMIC RESOLUTION OF SUBGROUP-SPECIFIC MEDULLOBLASTOMA ARCHITECTURE

Nicholas Willard[1], Kent Riemondy[2], Andrea Griesinger[2], Michael Kaufman[2], Sujatha Venkataraman[2], Nicholas Foreman[1], Rajeev Vibhakar[1], Andrew Donson[1]; [1]Children's Hospital Colorado, Aurora, CO, USA. [2]University of Colorado, Aurora, CO, USA

Despite a growing understanding and stratification of medulloblastoma, it remains an aggressive childhood brain tumor with high morbidity and mortality. Multimodal genomic and epigenomic analysis has permitted the classification of medulloblastoma into four subgroups with varying biology and clinical behavior: WNT, Sonic-Hedgehog (SHH), Group 3, and Group 4. In our previously published work, Single-cell RNA sequencing (scRNAseq) identified distinct tumor cell subpopulations in specific medulloblastoma groups. However, this technology is limited by its lack of architectural information. Spatial transcriptomics is a relatively new technology that permits the analysis of gene expression as it occurs within organized tissue. In our ongoing study, we utilized Visium spatial transcriptomics, integrated with scRNAseq data and immunohistochemistry, to analyze frozen samples of medulloblastomas (SHH, Group 4, and Group 3 with and without MYC amplification). In SHH in particular, we were able to identify scRNAseq

populations within the geographically constricted Visium data, including SHH-C2, a population located in histologic nodules, the predominant neuronal-differentiated population SHH-C1, and progenitor populations (SHH-B1 and B2). In addition, we were able to visualize clusters not detectable by scRNAseq – a cluster lining nodules with expression of vascular endothelium marker, reticulin and M2-macrophage genes, and a novel DNA-repair cluster. In addition, Visium data permits the spatial constraint of proliferating cells, which is frequently problematic in scRNAseq, as dividing cells cluster independently. The proliferation is highest in the SHH-B2 minor progenitor population, absent in the SHH-C1 major differentiated population, and is moderate in other population including the SHH-C2 nodules. Group 3 and 4 medulloblastoma are more complex but show preliminary corroboration with scRNAseq data. In summary, Visium allows us to map subpopulations identified by scRNAseq to tumor architecture more definitively and rapidly than IHC. These novel insights advance our understanding of medulloblastoma, a critical step in improving treatment options for children with this disease.

MEDB-45. FUNCTIONAL GENOMICS IDENTIFIES EPIGENETIC REGULATORS AS NOVEL THERAPEUTIC TARGETS FOR SONIC HEDGEHOG MEDULLOBLASTOMA

Foteini Tsiami[1], Federica Piccioni[2], David Root[2], Pratiti Bandopadhayay[3], Rosalind Segal[4], Ghazaleh Tabatabai[5], Daniel Merk[5]; [1]Department of Neurology & Interdisciplinary Neuro-Oncology, Hertie Insitute for Clinical Brain Research, University Hospital Tübingen, Eberhard Karls University Tübingen, Tuebingen, Germany. [2]The Broad Institute of MIT and Harvard, Cambridge, USA. [3]Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, USA. [4]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, USA. [5]Department of Neurology & Interdisciplinary Neuro-Oncology, Hertie Institute for Clinical Brain Research, University Hospital Tübingen, Eberhard Karls University Tübingen, Tuebingen, Germany

Medulloblastoma (MB) is among the most common malignant childhood brain tumors that comprises a group of four molecularly distinct diseases. A significant proportion of these tumors is characterized by aberrant activation of the canonical sonic hedgehog (SHH) signaling pathway. Although small-molecule inhibitors targeting Smoothened (SMO) have proven a promising treatment approach for SHH-MB subgroup, primary or acquired resistance impedes its clinical efficacy. Therefore, novel targeted approaches are urgently needed to improve therapeutic strategies for this tumor entity. Here, we conducted a genome-wide CRISPR/Cas9 knockout screen in a murine and a human SHH-MB cell line, SMB21 and DAOY, respectively, in order to decipher tumor-specific genetic dependencies. Our data demonstrate that SMB21 cells highly depend on positive regulators of the SHH pathway, such as Smo and Gli1 for their survival, as opposed to DAOY cells, suggesting that the latter does not represent a faithful model of SHH-MB. Members of the epigenetic machinery such as Dnmt1 and Smarca5 scored strongly as SMB21-context specific essentialities. Pharmacologically, we show that DNMT1 inhibition is efficacious at clinically relevant concentrations against SMO inhibitor- sensitive, as well as resistant SHH-MB cell lines, indicating novel therapeutic avenues for SHH-MB. By performing RNA sequencing of SMB21 cells, we identified early and late changes in global gene expression induced by DNMT1 inhibition, including decreased expression of mediators of SHH signaling, such as Gli1 and Gli2. Of note, gene set enrichment analysis revealed that DNMT1 inhibition downregulates top gene sets associated with cell cycle progression, corroborating the screening results that Dnmt1 is essential for SMB21 proliferation. Further global DNA methylation profiling in SMB cells will help to define the molecular basis of sensitivity to DNMT1 inhibitors in SHH-MB. Summarizing, our data highlight the potential of inhibitors targeting epigenetic regulators in SMO inhibitor- sensitive and resistant MB for more efficacious treatment options.

MEDB-46. ONC201 AFFECTS GROUP 3 MEDULLOBLASTOMA GROWTH BY IMPAIRING CANCER STEM CELLS

Luana Abballe[1], Celeste Antonacci[1], Matteo Gianesello[2], Chiara Lago[2], Francesca Nazio[1], Angela Mastronuzzi[1], Giuseppina Catanzaro[3], Angela Di Giannatale[1], Luca Tiberi[2], Franco Locatelli[1,4], Evelina Miele[1]; [1]Department of Pediatric Hematology/Oncology and Cellular and Gene Therapy, Rome[00165], Italy. [2]Armenise-Harvard Laboratory of Brain Cancer, Department CIBIO, University of Trento, Via Sommarive[9], [38123], Trento, Italy. [3]Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena [324], [00161], Rome, Italy. [4]Department of Pediatrics, Sapienza University of Rome, Rome, Italy

Cancer stem cells (CSCs) represent a sub-population of cancer cells capable of proliferating and generating heterogeneous cancer cell types. Acquisition of stemness features may represent a strong advantage for neoplastic cells to promote tumorigenesis and progression, driving resistance to conventional therapy and promoting disease relapse. CSCs have been discovered and isolated in major pediatric brain tumors, including medulloblastoma (MB), the most common solid malignancy in childhood. The unfolded protein response (UPR) represents an adaptation mechanism to metabolic obstacles in CSCs, able to increase tumor aggressiveness. The initial activation of the UPR is cytoprotective but the acute activation led to cell death. We found that UPR is active in MB stem cells (MBSC) and particularly in group 3 (G3). ONC201 is an imipridone compound that activates p53-independent apoptosis causing changes in gene expression similar to those caused by UPR. Here, we aim to test the in vitro efficacy of ONC201 on G3 MBSC. We selected 4 G3 MBSC (D341-Med, D283-Med, Med411, and CHLA-01-Med), for the in vitro study. Cells were choosen for their "fidelity" to the MB subgroup through the analysis of global methylation profiling, were grown in stemness conditions and expressed stemness markers at high levels. We investigated the efficacy of ONC201 treatment on CSC features, by evaluating cell viability, cell death, protein synthesis, self-renewal, and cell cycle. ONC201 treatment on G3 MB cells led to an upregulation of ATF4, a key molecule of the UPR, and the induction was stronger in MB cultured in a "stem-like" medium. Moreover, in the most MBSC analyzed, ONC201 was effective against CSCs whether by reduced cell viability, protein synthesis, and self-renewal. We also observed a trend of increased cell death. Our results suggest that ONC201 is potentially effective in treating G3 MB by compromising the stem cell compartment, and thus deserving further investigations.

MEDB-47. CD4+ T CELLS RESTRICT MEDULLOBLASTOMA GROWTH AND DISSEMINATION

Tanja Eisemann, Robert Wechsler-Reya; SBP Medical Discovery Institute, La Jolla, CA, USA

The immune system serves as a powerful defense not only against pathogens and parasites but also against neoplastic cells. Emerging immunotherapies that boost the activity of tumor-reactive immune cells or counteract immune suppressive mechanisms have shown promising effects in certain cancer types. However, the success of immunotherapy for brain tumors has been limited, highlighting the need for a better understanding of the immune microenvironment. Our preliminary studies have shown that T cells critically affect tumor growth in mouse models of the pediatric brain tumor medulloblastoma. In particular, depletion of CD4+ T cells results in more aggressive growth of medulloblastoma cells and allows these cells to metastasize to the spinal cord. To test whether CD4+ T cells can recognize and attack tumor cells directly, we generated MHC class II knockout tumors. Surprisingly, depletion of CD4+ cells still enhanced tumor growth and metastasis. These results suggest that CD4+ T cells regulate medulloblastoma growth independently of MHC II on tumor cells. We hypothesized that CD4+ T cell may not directly kill tumor cells but recruit and activate another effector immune cell type that eliminates tumor cells. As CD4+ T cells have a well-studied helper function for CD8+ T cells, we examined whether their anti-tumoral function relies on the activation of cytotoxic CD8+ T cells. The depletion of CD4+ T cells still resulted in advanced growth of MHC class I-deficient, and thus CD8+ T cell resistant, tumor cells indicating that CD4+ T cells counteract tumor growth in a CD8+ T cell-independent manner. Ongoing studies are aimed at elucidating the mechanisms by which CD4+ T cells regulate medulloblastoma growth, including the antigen-presenting cells that activate them and the effector cells responsible for killing tumor cells. These studies will advance our understanding of the immune microenvironment in medulloblastoma and allow us to design more effective therapies.

MEDB-48. INFANT MEDULLOBLASTOMA - SHH SUBTYPE – WITH RESIDUAL DISEASE. TO TREAT OR NOT TO TREAT

Christine Dahl, Sarita Depani, Kriti Hedges, Fernando Aguirregomezcorta, Kristian Aquilina, Owase Jeelani, Olivia Carney, Sniya Sudhakar, Ulrike Loebel, Felice d´Árco, Kshitij Mankad, Darren Hargrave, Mette Jorgensen; Great Ormond Street Hospital for Children, London, United Kingdom

Management of infant medulloblastoma remains a challenge. Front-line chemotherapy can successfully avoid radiation in low-risk infant medulloblastoma. Patients that do relapse can be salvaged long-term with radiotherapy. We report 4 cases of infants with medulloblastoma treated with chemotherapy (HIT2000 protocol) with residual or progressive disease. RESULTS: Four cases of infant medulloblastoma, all MBEN/nodular desmoplastic SHH type B, p53 WT, no MYC / MYCN amplification. CASE 1: 16 month old girl, metastatic lesions in the cerebellum and meningeal enhancement. Germline SUFU mutation. After 3 cycles of chemotherapy MRI showed more enhancement of the residual disease.