activation mechanisms by matched genome sequencing and DNA methylation profiling, respectively. Our findings will be applied to deconvolute bulk RNA sequencing data, thus identifying therapeutically relevant signaling networks in larger cohorts of medulloblastoma patients. Eventually, candidate targets will be validated on patient-derived cell models and xenografts by overexpression and inhibition studies. Together, here we aim at identifying tumor-driving receptor/ligand interactions in medulloblastoma, with the goal to define targets susceptible to precision oncology approaches.

MEDB-83. A NOVEL EPIGENETIC NANOTHERAPEUTIC STRATEGY TO INDUCE MEDULLOBLASTOMA DIFFERENTIATION

Praveen Raju[1], Daniel Tylawsky[2], Jake Vaynshteyn[1], Jeffrey Gerwin[1], Daniel Heller[2], Matija Snuderl[3]; [1]Icahn School of Medicine at Mount Sinai, New York, NY, USA. [2]Memorial Sloan-Kettering Cancer Center, New York, NY, USA. [3]NYU Langone Medical Center, New York, NY, USA

The histone-lysine N-methyltransferase EZH2 is the catalytic component of the PRC2 complex and is overexpressed in several medulloblastoma subtypes. However, its role in medulloblastoma tumorigenesis has been shown to be context-dependent using genetic approaches. Furthermore, pharmacological approaches have been limited by the very poor blood-brain barrier (BBB) penetration of current EZH2 inhibitors in use. Using laser capture microdissection and RNA-Seq analysis of human nodular/desmoplastic SHH medulloblastoma FFPE tissue, we provide data for the spatial epigenetic heterogeneity of primitive/proliferative regions compared to nodular/mature regions. Bioinformatic analysis identifies ~120 differentially expressed genes between primitive and mature regions with enrichment for genes regulated by H3K4me3 and H3K27me3 or SUZ12. ChIP-Seq analysis shows striking differences in H3K27me3 enrichment between primitive and mature medulloblastoma cells including at the EZH2 locus. Utilizing a genetically-engineered mouse model of SHH medulloblastoma, we show that conditional EZH2 genetic ablation within medulloblastoma cells results in wide-spread tumor cell differentiation (n=31 mice; *p=2e-07). Conversely, conditional EZH2 (Y641F) activation in this GEM model prevents tumor cell differentiation. Notably, we have found that the CDNK2A (p16) locus is an important EZH2 target that regulates tumor cell differentiation. qRT-PCR analysis of SHH medulloblastoma in wild-type and Ezh2 knockout settings show significant reduction in Gli1 and CCND1 and increase p15 and p16 expression in Ezh2 knockout mice compared to Ezh2 wildtype mice (*p<0.05). Importantly, genetic ablation of p16 conditionally in SHH MB EZH2 double knockout mice rescues the widespread tumor cell differentiation (n=9 mice; *p=3e-06) seen in Ezh2 single knockout SHH medulloblastoma mice. Finally, we developed a novel fucoidan-based nanoparticle strategy to deliver the EZH2 inhibitor (EPZ-6438) across the intact BBB of this GEM model to achieve significant extension of mouse survival (median 70 days compared to 19 days in control mice; *p=0.01, Mantel-Cox) with potential utility for other pediatric brain tumors.

MEDB-84. THE FRENCH EXPERIENCE OF ELP1-RELATED MEDULLOBLASTOMAS

Arnault Tauziède-Espariat[1], Léa Guerrini-Rousseau[2], Alexandre Perrier[3], Jacob Torrejon[4], Flavia Bernardi[4], Mathilde Filser[3], Pascale Varlet[1], Emilie De Carli[5], Anne Pagnier[6], Pierre Leblond[7], Cécile Faure-Conter[7], Francois Doz[8], Anne-Isabelle Bertozzi[9], Ludovic Mansuy[10], Marjolaine Willems[11], Gilles Palenzuela[12], Natacha Entz-Werle[13], Christine Bourneix[3], Lauren Hasty[1], Olivier Delattre[3], Thomas Blauwblomme[14], Kevin Beccaria[14], Alice Metais[1], Olivier Ayrault[4], Fabrice Chrétien[1], Franck Bourdeaut[8], Christelle Dufour[2], Julien Masliah-Planchon[3]; [1]Department of Neuropathology, GHU Paris, Sainte-Anne Hospital, Paris, France. [2]Department of Children and Adolescents Oncology, Gustave Roussy, Villejuif, France. [3]Laboratory of Somatic Genetics, Curie Institute Hospital, Paris, France. [4]Université Paris Sud, Université Paris-Saclay, CNRS UMR3347, INSERM U1021, Orsay, France. [5]Department of Pediatrics, CHU d'Angers, Angers, France. [6]Department of Pediatrics, CHU de Grenoble, Grenoble, France. [7]Institut d'hématologie et d'oncologie pédiatrique, Centre Léon Bérard, Lyon, France. [8]SIREDO Center Care, Innovation, Research In Pediatric, Adolescent and Young Adult Oncology, Curie Institute, Paris, France. [9]Department of Pediatrics, CHU de Toulouse, Toulouse, France. [10]Department of Pediatric onco-hematology, CHU de Nancy, Nancy, France. [11]Department of Genetic, CHU de Montpellier, Montpellier, France. [12]Department of Pediatrics, CHU de Montpellier, Montpellier, France. [13]Department of Pediatric onco-hematology, CHU de Strasbourg, Strasbourg, France. [14]Department of Pediatric Neurosurgery, Necker Hospital, Paris, France

Medulloblastoma (MB), the most frequent embryonic tumor of the cerebellum is classified into four molecular subgroups (WNT group, SHH group, group 3 and group 4). Although the vast majority of MB are sporadic, predisposing genetic diseases have been described in rare WNT MB and more frequently in the SHH group. In a recent pediatric series of SHH-MB, germline alterations of the ELP1 gene have been described in 14% of cases, making this gene the most frequent genetic predisposition in MB. We have investigated the potential interest of ELP1 immunostaining on a large cohort of 132 MB. A complete loss of ELP1 staining was observed in 12 SHH MB (among 57 total SHH MB: 21%). The loss of ELP1 immunostaining was well correlated with the presence of a bi-allelic alteration of the gene except for one case for which the MB had a loss of ELP1 protein expression demonstrated by immunohistochemistry (IHC) and confirmed by whole proteome analysis, although no obvious genetic alteration in the coding sequence of ELP1 could be found. Molecular analysis of a large "molecular" cohort of 266 MB from French centers for which somatic ELP1 was sequenced allows to identify 12 additional MB with bi-allelic ELP1 genetic alterations. Our results demonstrate the benefit of the ELP1 IHC as an accurate and reliable tool to screen ELP1-deficient MB. This new immunohistochemical tool will now be advantageously used to screen SHH MB upfront for genetic alteration in ELP1, and will subsequently help orientating these patients towards genetic counseling.

MEDB-85. TRANSCRIPTIONAL COMPLEXES AS RESISTANCE DRIVERS TO BET INHIBITION

Adam Boynton[1], Leslie Lupien[1], Rushil Kumbhani[1], Gabrielle Gionet[1], Madison Chacon[1], Amy Goodale[2], David Root[2], Hasmik Keshishian[2], Margaret Robinson[2], Steven Carr[2], Pratiti Bandopadhayay[1,2]; [1]Dana-Farber Cancer Institute, Boston, MA, USA. [2]Broad Institute of MIT and Harvard, Cambridge, MA, USA

BET-bromodomain inhibition (BETi) is a promising therapeutic strategy to target *MYC*-driven cancers, including Group 3 medulloblastoma, a deadly childhood brain tumor. We have shown that BET inhibitors exhibit preclinical efficacy against *MYC*¬-amplified medulloblastoma, providing motivation to evaluate this drug class in early phase clinical trials. However, our work has also found that *MYC*-amplified medulloblastoma cells can acquire resistance to BETi, suggesting that curative responses for this disease will require combination therapy. To guide the development of such combination therapies, we have focused our efforts on elucidating the mechanisms through which medulloblastoma cells acquire resistance to BETi. We found that medulloblastoma cells can develop tolerance to BETi by reinstating the expression of cell-essential "rescue genes," which include bHLH transcription factors, cell-cycle regulators, and anti-apoptosis genes. This transition to the resistant cell state is mediated through changes in chromatin structure including the upregulation of H3K4me3 promoters. Our preliminary results suggest that BETi-resistant cells maintain mRNA transcription and protein translation of important mediators of resistance. Importantly, we observe that BETi-resistant medulloblastoma cells are more dependent on specific protein complexes involved in transcriptional regulation. This project explores the mechanisms through which these transcriptional regulators help maintain transcription of rescue genes that drive BETi resistance and evaluates the potential of targeting these drivers of BETi resistance. These results will help guide the development of combination approaches to improve the efficacy of BETi for the treatment of *MYC*-driven medulloblastoma.

MEDB-86. A RE-INDUCTION REGIMEN FOR CHILDREN WITH RECURRENT MEDULLOBLASTOMA

Katrina O'Halloran[1], Sheetal Phadnis[2], Laura Metrock[2], Gregory Friedman[2], Tom Davidson[1,3], Nathan Robison[1,3], Girish Dhall[2], Ashley Margol[1,3]; [1]Children's Hospital Los Angeles, Los Angeles, California, USA. [2]Children's of Alabama, Birmingham, Alabama, USA. [3]Keck School of Medicine at University of Southern California, Los Angeles, California, USA

Medulloblastoma is the most common malignant brain tumor of childhood. Despite multi-modal therapies, ~30% of patients experience disease recurrence, which portends a poor prognosis. At initial recurrence, intensive chemotherapy may be effective prior to various consolidation therapies including high dose chemotherapy with autologous stem cell rescue or irradiation. We report outcomes for nine children treated at two institutions with the following regimen: cyclophosphamide 1500mg/m2/dose days 1,2; irinotecan 125mg/m2/dose days 1,8; temozolomide 150mg/m2/dose days 1-5, and oral etoposide 50mg/m2/dose days 1-7. Patients received 2-4 cycles based upon disease response and physician preference. The mean time from initial diagnosis to first recurrence was 19 months. After receiving two cycles of therapy, two patients had complete response (CR) and proceeded to consolidation. Of the remaining seven patients, five had partial response (PR) and two had stable disease (SD). Overall response rate was 78% after 2 cycles. Two patients with PR proceeded directly to consolidation with irradiation. Five patients (3 PR, 2 SD) received 2 additional cycles. After four cycles there was one CR, two with minimal residual disease, one SD and one progressive disease (PD). Four patients (44%) are alive with no evidence of disease (NED). One patient died of consolidation-related toxicity but had NED at time of death 28 months from initial recurrence. Five patients developed PD. Two patients died of disease, two are alive with disease, and one is alive with NED after PD and additional therapy. There were no treatment-related deaths. Infection was the most common com-

plication. Five patients had febrile neutropenia and two developed sepsis. One patient required dose reduction for prolonged thrombocytopenia. Peripheral blood stem cell collection was achieved in all patients for whom it was attempted. This re-induction regimen is generally well-tolerated and effective in inducing responses for children with recurrent medulloblastoma.

MEDB-87. TRANSCRIPTOME-DRIVEN DRUG REPURPOSING IN GROUP 3 MEDULLOBLASTOMA

David Doss[1], Ranjana Kanchan[2], Naveenkumar Perumal[2], Surinder Batra[2], Sidharth Mahapatra[2,3]; [1]Creighton University School of Medicine, Omaha, Nebraska, USA. [2]University of Nebraska Medical Center, Omaha, Nebraska, USA. [3]Children's Hospital and Medical Center, Omaha, Nebraska, USA

Across the molecular spectrum of medulloblastoma (MB), group 3 (G3) tumors are the most aggressive with <50% five-year survival, the lowest of all MB subgroups. G3 MB tumors are characterized by frequent metastases at diagnosis, unique methylation profiles, MYC amplification, and i17q, but these unique molecular features have yet to be exploited for therapeutic purposes despite their contribution to the disease process. As such, we sought to address this gap in survivorship by identifying FDA-approved compounds with the potential to inhibit cellular processes critical to G3 MB tumor proliferation and metastasis, aiming to exploit the unique molecular pathogenesis of G3 tumors. Guided by analysis of RNA-sequencing data from locally obtained, patient-derived MB samples against the LINCS chemical perturbagens database, we identified nortriptyline (NT), a tricyclic antidepressant, as a candidate MB therapeutic due to: 1) its ability to revert the transcriptomic signature of G3 MB to a normal cerebellum-like state and 2) its ability to cross the blood-brain barrier. We first identified the IC_{50} of NT in D425 and HDMB03 cells as 28μM and 20μM, respectively. Then, we observed that NT increased apoptosis of HDMB03 cells 3-fold by flow cytometry and confirmed our observations with Western blotting of apoptotic markers. Additionally, NT treatment resulted in abrogation of colony formation, impairment of wound healing, and inhibition of cell migration and invasion in vitro in HDMB03 cells. In all, transcriptome-driven drug repurposing holds great promise, as identifying novel uses for compounds with a known safety profile can deliver effective treatments into the hands of both patients and physicians in an expedited manner when compared to traditional means.

MEDB-88. BAF60C/SMARCD3-MEDIATED NOVEL NEURODEVELOPMENTAL EPIGENOMIC PROGRAM PROMOTES METASTATIC DISSEMINATION IN MEDULLOBLASTOMA

Han Zou[1,2], Bradley Poore[1], Emily Brown[3], Zhongliang Hu[4], Xuejun Li[2,5], Ian Pollack[1,6], Robert Friedlander[1], Sarah Hainer[3,7], Michael Taylor[8], Baoli Hu[1,6]; [1]Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA, USA. [2]Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha, Hunan, China. [3]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA. [4]Department of Pathology, Xiangya Hospital, Central South University, Changsha, Hunan, China. [5]Hunan International Scientific and Technological Cooperation Base of Brain Tumor Research, Changsha, Hunan, China. [6]John G. Rangos Sr. Research Center, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA. [7]UPMC Hillman Cancer Center, Pittsburgh, PA, USA. [8]Developmental & Stem Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada

Normal brain development relies on precise genetic and epigenetic spatiotemporal regulation of gene expression. How dysregulation of neurodevelopment relates to medulloblastoma, the most common pediatric brain tumor, remains elusive. Here, we uncovered a novel neurodevelopmental epigenomic program that regulates Purkinje cell migration in developing cerebellum is hijacked to induce tumor metastatic dissemination in medulloblastoma. Integrating publicly available datasets with our in-house data, unsupervised analyses revealed that BAF60C/SMARCD3, a subunit of SWI/SNF chromatin remodeling complex, promotes tumor cell migration in vitro and metastasis in vivo. Based on analyzing the single-cell RNAseq data of cerebellum developmental trajectory in mice and humans, aligning with the medulloblastoma patients' datasets, we found that BAF60C/SMARCD3 regulated DAB1-mediated Reelin signaling is involved in Purkinje cell positioning during cerebellum development and medulloblastoma metastasis by orchestrating the cis-regulatory elements (CREs) at the DAB1 gene locus. Analysis of spatiotemporal gene expression and chromatin architecture in the human and mouse cerebellum demonstrated that transcription activity of the BAF60C/SMARCD3-DAB1 circuit is downregulated in a mature state of cerebellar development, however, is upregulated in metastatic medulloblastoma. We further identified that a core set of transcription factors, enhancer of zeste homolog 2 (EZH2) and nuclear factor I X (NFIX), bi-directionally control BAF60C/SMARCD3 transcriptional regulation by coordinating with the CREs at the BAF60C/SMARCD3 gene locus to form a chromatin hub during developing cerebellar development and medulloblastoma metastatic dissemination. Highly expressed BAF60C/SMARCD3 activates the Reelin/DAB1 signaling pathway downstream Src kinase, which was validated in the pair-wised primary and metastatic tumors from medulloblastoma patients. Preclinical medulloblastoma mouse models revealed that inhibiting Src activity reduces tumor cell migration and metastatic dissemination at a lower and safe dose. Together, these data deepen our understanding of how the developmental program influences disease progression and provide an opportunity for the development of therapeutics for this devastating brain cancer in children.

MEDB-89. ELUCIDATION OF THE ONCOGENIC ROLE OF NUCLEAR FACTOR I/B (NFIB) IN GROUP 3 MEDULLOBLASTOMA

Naveenkumar Perumal[1], Ranjana Kanchan[1], David Doss[2], Ishwor Thapa[3], Mohd W Nasser[1], Surinder Batra[1], Sidharth Mahapatra[1]; [1]University of Nebraska Medical Center, Omaha, NE, USA. [2]Creighton University School of Medicine, Omaha, NE, USA. [3]University of Nebraska Omaha, Omaha, NE, USA

Amongst the 4 subgroups of medulloblastoma (MB), tumors falling into group 3 are the most aggressive and associated with increased incidence of aberrations on chromosome 17p, c-Myc amplification, metastases at diagnosis, and rapid tumor relapse. Thus, patients with group 3 tumors suffer the worst prognosis with a 5-year survival rate of <50%. We have prior identified a novel tumor-suppressive microRNA, miR-212, silenced on chromosome 17p and its deregulated oncoprotein target, Nuclear Factor I/B (NFIB). Here, we sought to identify the role of NFIB in group 3 MB pathophysiology. NFIB is a transcription factor that regulates chromosomal gene accessibility and expression of pro-metastatic genes in various cancers. Transcriptomic interrogation of group 3 tumors revealed deregulated expression of NFIB. Kaplan-Meier survival analysis confirmed poorer survival in NFIB high-expressing patients. Using inducible silencing of NFIB in a classic group 3 MB cell line, HDMB03, we observed downregulation of key driver genes (49 genes, Log2 fold change < -0.5, $p < 0.001$) associated with group 3 MB pathogenesis by RNA sequencing. NFIB expression knockdown ($NFIB^{KD}$) further reduced tumor cell growth and aggressiveness, as evidenced by reduced proliferation, colony formation, migration, and invasion. $NFIB^{KD}$ also affected group 3 MB stemness, with attenuation of medullospheres and a reduction in stem cell markers (Nanog, Oct4, Sox2, CD133). Moreover, $NFIB^{KD}$ destabilized c-Myc phosphorylation at serine-62, resulting in reduced total c-Myc levels and subsequent cellular apoptosis. Concurrently, $NFIB^{KD}$ decreased the expression of upstream activators of c-Myc such as p-Akt and p-Erk. Taken together, these results validate the oncogenic role of NFIB in group 3 medulloblastomas and provide a potential new therapeutic target.

MEDB-90. IRON IMBALANCE CAN POTENTIATE CISPLATIN RESPONSE IN PEDIATRIC MEDULLOBLASTOMA BY REGULATING FERROPTOSIS

Ranjana Kanchan[1], Naveenkumar Perumal[1], Parvez Khan[1], David Doss[1,2], Ramakanth Chirravuri Venkata[1], Ishwor Thapa[3], Raghupathy Vengoji[1], Jyoti Kaushal[1], Jawed Siddiqui[1], Mohd. Wasim Nasser[1], Surinder Batra[1], Sidhartha Mahapatra[1,4]; [1]University of Nebraska Medical Center, Omaha, NE, USA. [2]Creighton University, Omaha, NE, USA. [3]University of Omaha, Omaha, NE, USA. [4]Children's Hospital and Medical Center,, Omaha, NE, USA

Medulloblastoma (MB), the most common malignant pediatric brain tumor, is a leading cause of childhood mortality. Of the four primary subgroups, patients with group 3 tumors have the poorest prognosis. Loss of chromosome 17p is a high-risk feature associated with poor outcomes in group 3 tumors. We recently elucidated the tumor suppressive properties of a novel miR, miR-1253, on the terminal end of 17p. In further exploring its anti-neoplastic effects, we discovered that miR-1253 can disrupt iron homeostasis, causing oxidative stress and inducing lipid peroxidation. These concurrent events are capable of triggering an iron-mediated form of cell death called ferroptosis. Notably, our in silico interrogation of ferroptosis regulator genes (FRGs) in group 3 tumors revealed high expression of genes associated with iron transport and glutathione metabolism. These included mitochondrial iron transporters and GPX4, a critical regulator of ferroptosis. Restoration of miR-1253 expression in group 3 cell lines resulted in specific downregulation of ABCB7, an iron-sulfur cluster exporter, and GPX4. Consequently, cytosolic and mitochondrial labile iron pools rose, glutathione levels declined, and mitochondrial oxidative stress and lipid peroxidation were induced. These events were recapitulated by ABCB7 knockdown and potentiated cell death. Treating miR-1253-expressing cancer cells with cisplatin, a group 3 MB chemotherapeutic agent with ferroptotic properties, further elevated oxidative stress, depleted glutathione levels, and augmented lipid peroxidation, with added inhibitory effects on cell viability and colony formation. Treatment with a ferroptosis inhibitor (ferrostatin-1) lead to recovery from the cytotoxic effects of this combination therapy. Our studies highlight a novel mechanism for group 3 MB pathogenesis via ferroptosis regulation and provide a proof-of-concept for exploiting group 3 MB tumor vulnerability to iron imbalance as a novel treatment strategy.