Genetically engineered mouse models and cross-species transcriptomics have provided mounting evidence of discrete, subgroup-specific developmental origins. Likewise, murine single-cell transcriptional atlases of cerebellar development have repeatedly provided further clues into MB subgroup origins, particularly for poorly defined Group 3 and Group 4 MB. However, initial studies were underpowered to characterize rare populations and lacked robust validation, resulting in incomplete findings. Herein, we leveraged a novel murine cerebellar model to perform high-throughput and integrative multi-omic strategies to deeply dissect MB origins. Isolation of spatially and temporally discrete developmental trajectories of key glutamatergic lineages born out of the murine upper rhombic lip provided an enhanced reference for mapping MB subgroup origins, especially for Groups 1 and 4-MB. However, human-specific anatomic and cellular complexity, particularly within the rhombic lip germinal zone complicated murine-derived inferences. Further tumor-normal integrations using a novel single-cell atlas of the human fetal cerebellum, accompanied with laser-capture micro-dissected transcriptional and epigenetic datasets, reinforced developmental insights extracted from candidate murine cerebellar lineages. Characterization of compartment-specific transcriptional signatures identified in the human upper rhombic lip implicated convergent cellular correlates of Group 3 and Group 4-MB, suggestive of a common developmental trajectory underlying their ancestry. Systematic imaging review and 3D summarization of a large clinical trial series of patient tumors, coupled with our advanced insights into developmental signatures, substantiated subgroup-specific patterns observed in our MB models. Together, these strongly implicate a common lineage trajectory of the upper rhombic lip as the probable origin of Group 3 and Group 4-MB. These important findings provide unprecedented opportunities to explore context-dependent mechanisms of MB pathogenesis and will foster generation of improved preclinical models that more faithfully recapitulate tumor biology.

MBED-79. MYC-DRIVEN UPRGULATION OF THE DE NOVO SERINE AND GLYCINE PATHWAY IS A NOVEL THERAPEUTIC TARGET FOR GROUP 3 MYC-AMPLIFIED MEDULLOBLASTOMA Magaretta Adiamah,1 Janet C. Lindsay,1 Florence Burré,1 Sarah Kohe,1 Adikute Morel,1 Helen Blake,1 Rebecca M. Hill,1 Stephen Crosser,1 Tong Zhang,1 Oliver Maddocks,2 Andrew Peet,3 Louis Chesler,1 Ian Hickson1, Ross Maxwell,2 Steven C. Clifford,1 1Newcastle University Centre for Cancer, Newcastle University, Newcastle, United Kingdom, 2Institute of Cancer and Anticancer Therapy, University of Birmingham, Birmingham, United Kingdom, 3Division of Clinical Studies, Institute of Cancer Research, London, United Kingdom, 4Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom

Despite advances in the molecular sub-classification and risk-stratification of medulloblastoma (MB), a subset of tumours remain refractory to current multimodal therapeutics. Group 3 (MB3) Group 3 MBs represent around 25% of MBs, and amplification and elevated expression of MYC in this group correlate with poor survival outcomes. Since direct targeting of MYC remains refractory, an alternative, understanding and exploiting metabolic dependencies in MYC-amplified MB3 may reveal novel therapeutic opportunities. We engineered three independent regulatable MYC-amplified MB3 (MB3-R) cell-based models, each harbouring different human-MYC shRNA silencing control. In all three models, MYC knockdown (KD) revealed persistent MYC-dependent cancer phenotypes, reduction in proliferation and cell cycle progression. We utilised 1H high-resolution magic angle spectroscopy (HRMAS) and stable isotope-resolved metabolomics to assess changes in intracellular metabolites and pathway dynamics when MYC expression was modulated. Profiling revealed consistent MYC-dependent changes in metabolite concentrations across models. Notably, glycine was consistently accumulated following MYC KD suggesting altered pathway dynamics. 13C-glucose tracing further revealed a reduction in glucose-derived serine and glycine (de novo synthesis) following MYC KD which was attributable to lower expression of PHGDH associated with MYC amplification and poorer survival outcomes. MYC expressing cells showed greater sensitivity to pharmacological inhibition of PHGDH compared to MYC KD (MB3-R) and MB3 shRNA subgroup cell lines in vitro. Critically, targeting PHGDH in vivo, using MYC-dependent xenografts and genetically engineered mouse models, consistently slowed tumour progression and increased survival. In summary, metabolic profiling has uncovered MYC-dependent metabolic alterations and revealed the de novo serine/glycine synthesis pathway as a novel and clinically relevant therapeutic target in MYC-amplified MB3. Together, these findings reveal metabolic vulnerabilities of MYC-amplified MB3, which represent novel therapeutic opportunities for this poor-prognosis disease group.

MBED-80. CDK8 PROMOTES STENNESS OF MYC-DRIVEN MEDULLOBLASTOMA Dong Wang,1 Bethany Veo,1 Angela Pierce,1 Sujatha Venkataraman,1 Rajeev Vibhakar2; University of Colorado Anschutz Medical School, Aurora, CO, USA; 1University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Cyclin-dependent kinase 8 (CDK8) belongs to the transcription-related cyclin-dependent protein kinase family, CDK8 and cyclin C associate with the mediator complex to regulate gene transcription. Although CDK8 has been shown to be implicated in the malignancy of several types of cancer, its functional role in medulloblastoma remains largely unexplored. Here, we demonstrate how CDK8 plays an essential role in maintaining stemness and tumorigenesis in medulloblastoma stem cell. CDK8 inhibition suppresses stem cell-associated signaling in medulloblastoma cells and inhibits tumor cell self-renewal. Additionally, CDK8 is amplified in MYC-driven medulloblastoma, as positively correlated with c-MYC expression in human medulloblastoma specimens and associates with poor survival in patients. Using cut&sson assay, we found CDK8 associates with MED1 to activate transcription of MYC target genes. CDK8 attributes to MYC-driven transcriptional programs mediating DNA repair. Pharmacologic inhibitors and genetic depletion result in cessation of tumor growth in xenograft mouse models and increase in apoptosis and DNA damage. Collectively, our studies establish the selective inhibition of CDK8 inhibition as a viable therapeutic strategy in MYC-driven medulloblastoma.

MBED-81. COMBINED INHIBITION OF CDK11 AND EZH2 RESULTS IN REVERSION OF MYC-AMPLIFIED MEDULLOBLASTOMA Dong Wang,1 Bethany Veo,1 Angela Pierce,1 Sujatha Venkataraman,1 Rajeev Vibhakar; University of Colorado Anschutz Medical Campus, Aurora, CO, USA

We explored an shRNA library screen on 20 cyclin-dependent kinases to establish cyclin-dependent kinase 11 (CDK11) as a critical mediator in MYC-driven medulloblastoma. The effect and molecular mechanism of CDK11 in the proliferation and growth of medulloblastoma was investigated in vitro. Pharmacologic inhibitors and genetic depletion of CDK11 revealed a critical role in suppression of tumor growth in xenograft mouse models. Through combination chemical screening, we identified that 5-FU enhanced the apoptosis which induced by inhibition of CDK11 in medulloblastoma cells. In addition, we found CDK11 is a significant candidate kinase participating in the negative control of Wnt/β-catenin signaling. Down-regulation of CDK11 led to the accumulation of Wnt/β-catenin signaling receptor complexes through activation of transmembrane Frizzled (FZD) receptors which is suppressed by CDK11. We revealed that MYC-associated Cdka8 kinase regulate a common set of genes. Lack of Cdka8 and Cdk11 impaired Ezh2 recruitment and the establishment of histone H3 lysine 27 tri-methylation. We concluded that combined EZH2 and CDK11 inhibitors treatment concurrently activated Wnt signaling may be an effective treatment for Group 3 medulloblastoma.

MBED-82. EXPLORING CELL-CELL COMMUNICATION NETWORKS IN MEDULLOBLASTOMA USING SINGLE-CELL GENOMICS Lasa Gaehler1, Johannes Gojo2, Kyle S. Smith3, Laure Bihanac1, Marrella G. Filbin2,4, Paul A. Northcott5, Walter Berger1, Wolfram Hovestad1,2,4, Department of Pediatric Oncology, Dana-Farber Children’s Cancer and Blood Disorders Center, Boston, MA, USA; 2Broad Institute of Harvard and MIT, Cambridge, MA, USA; 3Department of Pediatrics and Adolescent Medicine and Comprehensive Center for Pediatrics, Medical University of Vienna, Vienna, Austria; 4Department of Developmental Neurobiology, St Jude Children’s Research Hospital, Memphis, TN, USA; 5Center for Cancer Research and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; 6Broad Institute of Harvard and MIT, Boston, MA, USA

Medulloblastoma is a high-risk embryonal brain tumor arising in the cerebellum. Genomic profiling has revealed a striking molecular heterogeneity between medulloblastoma patients, yet treatment regimens are mostly uniform. Many children with medulloblastoma die from their disease and surviving patients often face severe long-term side effects, highlighting an urgent need for more effective treatment options. We and others have recently identified potent intratumoral heterogeneity and defined cellular hierarchies within medulloblastoma tumors. The functional role of these cellular hierarchies remains unknown. We now hypothesize the existence of an inter-cellular communication network that is maintained by receptor/ligand interactions. To test our hypothesis, we use our medulloblastoma single-cell RNA sequencing dataset of 25 patients, as well as bulk RNA sequencing, DNA methylation array, and genome sequencing data across molecular subtypes. Single-cell RNA sequencing data are analyzed to dissect cell compartmentalization characterized by high expression of potentially oncogenic receptors and their respective ligands. Consequently, cell type-specific roles in auto- or paracrine signal transduction within the cellular community are explored. We further investigate downstream oncogenic signaling pathways by approximating transcription factor activity and explore genetic and epigenetic
activation mechanisms by matched genome sequencing and DNA methyla-
tion profiling, respectively. Our findings will be applied to deconvolute bulk RNA sequencing data, thus identifying therapeutically relevant signaling networks and in larger cohorts of medulloblastoma patients. 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 nAnoThERAPY STRATEGy TO INDUCE MEDULLOBLASTOMA DIFFERENTIATION

Praeven Raji1, Daniel Telzak2, Jake Yavnehstien2, Jeffrey Gerwin2, Daniel Heller2, Matija Smuder1; 1Icahn School of Medicine at Mount Sinai, New York, NY, USA. 2Memorial Sloan-Kettering Cancer Center, New York, NY, USA. *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 ap-
proaches have been limited by the very poor blood-brain barrier (BBB) penetra-
tion of current EZH2 inhibitors in use. Using laser capture microdissection and RNA-Seq analysis of human nodular/desmoplastic SHH medulloblastoma FFPE tissues, we were able to provide data for the spatial epigenetic heterogeneity of primitive/prolif-
erative regions compared to nodular/mature regions. Bioinformatic analysis iden-
tifies ~120 differentially expressed genes between primitive and mature regions with enrichment for genes regulated by H3K4me3 and H3K27me3 or SUZ12. Genes in this group are striking differences between primitive and mature medulloblastoma cells including at the EZH2 locus. Utiliz-
ing 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(-7)). Conversely, conditional EZH2 (Y414F) activation in this GEM model prevents tumor cell differen-
tiation. Notably, we have found that the CDN2K2a (p16) locus is an im-
portant EZH2 target that regulates tumor cell differentiation. qRT-PCR analysis of EZH2 in wild-type and mutant tumor cells demonstrated sub-
stantial reduction in G1 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 results in the tumor cell differentiation (n=9 mice; p=0.06) seen in EzH2 single knockout SHH medulloblastoma mice. Finally, we developed a novel fucosidase-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<0.001, Mantel-Cox) with potential utility for other pediatric brain tumors.

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

Arnaud Taüzié-Espariat1, Léa Guérin-Rousseau2, Alexandre Perrier2, Jacob Torrejon3, Flavia Bernardi1, Mathilde Fiser1, Pascale Varlet1, Emilie De Carli1, Anne Pagnier4, Pierre Leblic4, Cécile Faure-Conter5, Daniel Telzak2, Pascale Perrier2, Ludovic Mansuy3,3, Marjolaine Willems3, Gilles Palenzuela1, Natacha Entz-Werle1,1, Christine Bourrèux3, Lauren Hasty1, Olivier Delattre3, Thomas Blauwblomme4, Kevin Beccara5, Alice Metrais1, Olivier Ayraud3, Fabrice Christen4, Francck Bourrouilh2, Christelle Dutour1, Julien Masliah-Planchon6; 1Department of Neuropathology, GHU Paris, Sainte-Anne Hospital, Paris, France. 2Department of Children and Adolescents Oncology, Gustave Roussy, Villejuif, France. 3Laboratory of Somatic Genetics, Curie Institute Hospital, Paris, France. 4Université Paris Sud, Université Paris-Saclay, CNRS UMR1347, INSERM U1051, Orsay, France. 5Department of Pediatrics, CHU d’Angers, Angers, France. 6Department of Pediatrics, CHU de Grenoble, Grenoble, France. 7Institut d’hématologie et d’oncologie pédiatrique, Centre Léon Bérard, Lyon, France. 8SIREDO Center Care, Innovation, Research in Pediatrics, Adolescent and Young Adult Oncology, Curse Institute, Paris, France. 9Department of Pediatrics, CHU de Toulouse, Toulouse, France. 10Department of Pediatrics onco-hematology, CHU de Nancy, Nancy, France. 11Department of Genetic, CHU de Montpellier, Montpellier, France. 12Department of Pediatrics, CHU de Montpellier, Montpellier, France. 13Department of Pediatric onco-hematology, CHU de Strasbourg, Strasbourg, France. 14Department of Pediatric Neurosurgery, Necker Hospital, Paris, France

Medulloblastoma (MB), the most frequent embryonic tumor of the cere-
bral hemispheres, is one of the four molecular subgroups, SHH group, group 3 and group 4). Although the vast majority of MB are driven by progressive driver gene mutations, a minority of MB harbors 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 ELPI1 immunostaining on a large cohort of 132 MB. A complete loss of ELPI1 staining was observed in 12 SHH MB (among 57 total SHH MB: 21%). The loss of ELPI1 immunostaining was well correlated with the presence of a bi-allelic alteration. 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-85. TRANSCRIPTIONAL COMPLEXES AS RESISTANCE DRIVERS TO BET INHIBITION

Adam Boynton1, Leslie Lupin1, Rushil Kumbhari2, Gabrielle Gioner1, Madam Chacon3, Amy Goodale2, Davide Kno1, Hasnuk Keshishian2, Margaret Robinson2, Steven Carr2, Pratiti Bandopadhyay1,2, 3Dana-Farber Cancer Institute, Boston, MA, USA. 2Broad 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, which is promising motivation to evaluate this drug class in early phase clinical trials. However, we have also found that MYC-amplified medulloblastoma cells can acquire resistance to BETi, suggesting that curative responses for this disease will require BETi. To guide the development of such combina-
tion 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 reexpressing the expression of cell-essential “rescue genes,” which include important mediators of resistance 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 BET-resistant cells maintain mRNA transcription and protein translation of important mediators of resistance. Importantly, we observe that BET-resistant medulloblastoma cells are more dependent on specific protein complexes involved in transcriptional regulation. This project ex-
plores the mechanisms through which these transcriptional regulators help maintain transcription of rescue genes that drive BET resistance and evalu-
ates 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’Halloran1, Sheela Davidson1, Laura Metrock2, Gregory Friedman3, Tom Davidson1,4, Nathan Robison1,5, Girish Dhall3, Ashley Margol1,2, 1Children’s Hospital Los Angeles, Los Angeles, California, USA. 2Children’s of Alabama, Birmingham, Alabama, USA. 3Keck School of Medicine at University of Southern California, Los Angeles, California, USA

Medulloblastoma is the most common malignant brain tumor of child-
hood. Despite multi-modal therapies, ~30% of patients experience disease recurrence, which portends a poor prognosis. At initial recurrence, inten-
sive chemotherapy may be effective prior to various consolidation therapies including high dose chemotherapy with autologous stem cell rescue or ir-
radiation. 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 1-3, temozolomide 150mg/m2/dose days 1-5, and oral etoposide 30mg/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 two PD. Four WNT group patients were 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-