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 recently 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 large harmonized murine cerebellar atlas, targeted lineage enrichment, 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 Group 3 and Group 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, companioned 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 tumor location patterns observed at diagnosis. Together, our results 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.

MEDB-79. MYC-DRIVEN UPREGULATION OF THE *DE NOVO* SERINE AND GLYCINE PATHWAY IS A NOVEL THERAPEUTIC TARGET FOR GROUP 3 MYC-AMPLIFIED MEDULLOBLASTOMA

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Despite advances in the molecular sub-classification and risk-stratification of medulloblastoma (MB), a subset of tumours remain refractory to current multimodal therapies. Group 3 (MB_{Group3}) patients represent around 25% of MBs, and amplification and elevated expression of *MYC* in this group correlates with dismal clinical outcomes. Since direct targeting of MYC remains elusive, understanding and exploiting metabolic dependencies in MYC-amplified MB_{Group3} may reveal novel therapeutic opportunities. We engineered three independent regulable *MYC*-amplified MB_{Group3} cell-based models, each harbouring doxycycline-inducible anti-MYC shRNAs (two independent species) or a non-silencing shRNA control. In all three models, MYC knockdown (KD) revealed persistent MYC-dependent cancer phenotypes, reduction in proliferation and cell cycle progression. We utilised ^{1}H 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. ^{13}C-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, the rate-limiting enzyme of this pathway. Furthermore, in human primary tumours, elevated expression of PHGDH was associated with *MYC* amplification and poorer survival outcomes. MYC expressing cells showed greater sensitivity to pharmacological inhibition of PHGDH compared to MYC KD (MB_{Group3}) and MB_{SHH} 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 MB_{Group3}. Together, these findings reveal metabolic vulnerabilities of *MYC*-amplified MB_{Group3} which represent novel therapeutic opportunities for this poor-prognosis disease group.

MEDB-80. CDK8 PROMOTES STEMNESS OF MYC-DRIVEN MEDULLOBLASTOMA

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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 and mechanism in medulloblastoma remains largely unknown. Here, we demonstrate how CDK8 plays an essential role in maintaining stemness and tumorigenicity 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, is positively correlated with c-MYC expression in human medulloblastoma specimens and associates with poor survival in patients. Using cut&run assay, we found CDK8 associates with MED1 to activate transcription of MYC target genes. CDK8 attributes to MYC-driven transcriptional programs mediating DNA repair. Pharmaceutic 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.

MEDB-81. COMBINED INHIBITION OF CDK11 AND EZH2 RESULTS IN REGRESSION OF MYC-AMPLIFIED MEDULLOBLASTOMA

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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 were investigated in vitro. Pharmaceutic inhibitors and genetic depletion of CDK11 resulted in cessation 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/b-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 H3K27Me3. RNASeq and cut&run revealed that Cdk11 and mediator associated Cdk8 kinase regulate a common set of genes. Lack of Cdk8 and Cdk11 impaired Ezh2 recruitment and the establishment of histone H3 lysine 27 tri-methylation. We concluded that combined EZH2 and CDK8/CDK11 inhibitors treatment concurrently activated Wnt signaling may be an effective treatment for Group 3 medulloblastoma.

MEDB-82. EXPLORING CELL-CELL COMMUNICATION NETWORKS IN MEDULLOBLASTOMA USING SINGLE-CELL GENOMICS

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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 pronounced intra-tumoral 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 compartments 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 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

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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

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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

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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

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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-