MBRS-62. CURAXIN CBL0137 INHIBITS THE VIABILITY OF CANCEROUS CELLS IN PRE-CLINIC MODELS OF MYC-AMPLIFIED MEDULLOBLASTOMA

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Medulloblastoma is a malignant brain tumour that mostly occurs in children, and MYC-amplified medulloblastoma is characterized by pronounced invasiveness and dismal prognosis. There is no effective treatment for Medulloblastoma and its precise pathological mechanism remains obscure. Previous studies indicated that the altered epigenetic machinery manifested in the neoplastic transformation of MYC-amplified MB has become increasingly evident. It is hypothesized that epigenetic genes dependencies associated with small molecule inhibitors that have been approved or are in advanced development may help identify the potential therapeutic targets. By integrating mRNA expression profiles and the corresponding clinic-pathologic information of patients suffering from medulloblastoma, and analysing prior CRISPR screening results, we demonstrated that SSRP1 is a negative prognostic factor that functions to stimulate the viability of MB cells. SSRP1 is a subunit of the FACT complex, an important histone chaperone required for transcriptional regulation, DNA replication and damage repair. Its biological effect on tumour proliferation was assessed by using RNA interference and administering CBL0137, a small molecular inhibitor of FACT. Gene expression analysis also demonstrated that CBL0137 selectively downregulated the expression of MYC and NEUROD1. Furthermore, the administration of CBL0137 suppressed tumour growth in mouse xenograft models. This pharmacological method to selectively target MYC transcription was demonstrated in our study, and therefore can be applied as a promising treatment strategy for MYC-amplified medulloblastoma. In Conclusion, we identified an attractive strategy of selectively downregulating MYC transcription by applying inhibitor CBL0137, thereby revealing the potential clinical utility of CBL0137 to improve the prognosis of MYC-amplified medulloblastoma patients.

MBRS-63. THE ROLE OF THE SWI/SNF COMPLEX SUBUNIT SMARCD3 IN MEDULLOBLASTOMA

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Medulloblastoma is the most common malignant brain tumor in children, with the Group 3 (G3) having the worst prognosis of the subgroups (WNT, SHH, and Group 4). We aimed to determine the underlying differences between G3 and the other subgroups, with an emphasis on genes that control the epigenome for developing effective treatments for patients with this disease. To this end, we found that G3 has elevated expression of the SWI/SNF subcomponent, SMARCD3 (P<0.001), which serves to guide the SWI/SNF complex to different genomic regions through interactions with various transcription factors. However, little is known about function of SMARCD3 in cancer, particularly in medulloblastoma. Clinically, elevated SMARCD3 mRNA resulted in a poorer prognosis in medulloblastoma patients (P<0.0001), which was further validated in 63 patient tumors by immunohistochemical staining for SMARCD3. Interestingly, tumors that had metastasized often had elevated expression of SMARCD3, in all subgroups of medulloblastomas (P<0.0001) and G3 only (P<0.01) based on analyzing multiple published databases. An orthotopic mouse model further supported that SMARCD3 is highly expressed in metastatic tumors compared to primary tumors. Importantly, CRISPR-CAS9-mediated SMARCD3 deletion decreased cell migration in medulloblastoma cell lines. Mechanistically, SMARCD3 deletion led to decreased H3K27me3, suggesting that SMARCD3 may cooperate with PRC2 in regulation of gene expression. Together, our results indicate that SMARCD3 plays a significant role in the development of metastatic dissemination in medulloblastoma, especially in the G3 subgroup. Thus, targeting the SMARCD3-containing SWI/SNF Complex may effectively prevent tumor dissemination and improve clinical outcomes in children with medulloblastoma.

MBRS-64. STUDY OF ARGININE METHYL TRANSFERASES IN MEDULLOBLASTOMA

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Medulloblastoma is the most common malignant pediatric cancer and a leading cause of childhood cancer-related mortality. There is dire need for new therapies. Molecular sub-classification of medulloblastomas has identified chromatin modifiers as potential drivers of tumorigenesis. Expression of the *RE1* Silencing Transcription Factor (REST), a hub for assembly of repressive chromatin remodelers and a known regulator of neurogenesis, is elevated in a subset of human sonic hedgehog (SHH) subgroup medulloblastomas, and is associated with poor prognosis. Using a novel transgenic mouse model, we showed REST to be a driver of medulloblastoma development. Surprisingly, our studies also revealed a role for REST in promoting proliferation of granule cell progenitors (GCPs), the cells of origin of SHH-driven medulloblastoma, and a concomitant loss of telomeres and increased genomic instability. We performed a gain of function screen using a library of chromatin modifiers to understand the mechanism by which proliferative potential was maintained, despite the loss of telomeres. This screen identified the Protein Arginine Methyltransferase 6 (*PRMT6*) as a high confidence hit. PRMT6 upregulation caused a reduction in CDKN2A, an important regulator of replicative senescence. Evasion of senescence is frequently implicated in tumor progression. Using a chemical screen, we also identified a novel, selective, reversible and competitive inhibitor of PRMT6. The consequence of genetic and pharmacological inhibition of PRMT6 on cell proliferation and senescence will be reported. Thus, our studies are the first to demonstrate a role for arginine methyl transferase family of chromatin modifiers in medulloblastoma genesis.

MBRS-65. FBXW7 ACTS A TUMOR SUPPRESSOR IN MYC-DRIVEN MEDULLOBLASTOMA BY CONTROLLING A FEED-FORWARD REGULATORY LOOP OF PLK1 AND MYC

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Group 3 medulloblastoma (MB) is often accompanied by MYC amplification and has a higher rate of metastatic disease. So, it is critical to have more effective therapies for high MYC expressing sub-groups. Here we report that FBXW7, a substrate recognition component of the SKP1-CUL1-Fbox (SCF) E3 ligase, interacts with and targets c-MYC for polyubiquitination and proteasomal degradation. FBXW7 shows lower expression level in MYC-driven MB compared with other MB subgroups suggesting activity as a tumor suppressor. Genomic deletion or mutation of *Fbxw7* has frequently been identified in many human cancers but not in MB. We demonstrate that overexpression of *Fbxw7* in MB cells induces apoptosis and suppresses proliferation *in vitro* and *in vivo*. Both phospho-deficient (T205A) and phosphomimetic aspartic acid (T205D) mutants deactivate its tumor suppressor function suggesting a conformational change of its protein structure. Mechanistically, PLK1 kinase specifically phosphorylates FBXW7 and promotes its auto-polyubiquitination and proteasomal degradation, counteracting FBXW7-mediated degradation of oncogene substrates, including c-MYC and PLK1. Chip-Seq results show stabilized c-MYC in turn directly activates PLK1 and FBXW7 transcription, constituting a feedforward regulatory loop. Co-immunoprecipitation demonstrates that FBXW7 directly binds to PLK1 and c-MYC, facilitating their protein degradation by promoting the ubiquitination of both proteins. Furthermore, we show that FBXW7 protein can be stabilized by various kinase inhibitors, proposing a mechanism of kinase-targeted agents to treat MYC-driven MB. These results collectively demonstrate how kinase inhibition stabilizes the tumor suppressor FBXW7 in MYC-driven MB, thus revealing an important function of FBXW7 in suppressing MB progression.

MBRS-66. COST-EFFECTIVE METHOD TO INCORPORATE MOLECULAR CLASSIFICATION OF MEDULLOBLASTOMA INTO A LATIN-AMERICAN CLINICAL TRIAL

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BACKGROUND: It is now widely accepted that medulloblastoma actually comprises four distinct molecular subgroups, requiring specific treatment strategies. Implementing routine subgrouping in a time and cost effective manner is a major challenge in Latin America, particularly the development of molecular informed clinical trials. Herein we describe the feasibility of reliable and rapid molecular stratification using a qPCR method integrated with immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH – c-myc, monosomy 6) from heterogeneously fixed, low-quality FFPE samples across Latin America. RESULTS: Fifty-four FFPE samples were classified according to histologic and molecular criteria. Classic medulloblastoma was found in 53.7%, desmoplastic/extensive nodularity in 24.1%, NOS in 16,7% and anaplastic in 5,6%. IHC

markers classified patients in three groups (WNT, SHH and non-WNT/non-SHH) in 98% of cases. PCR-based method confirmed results from IHC in 81,5%. Additionally, we were able to detect WNT activation in 2 patients, previously classified as SHH. For both cases, the presence of monosomy 6 further confirmed WNT subgroup. Integration of these three techniques resulted in the following frequencies: WNT (13.0%), SHH (38.9%), group 3 (9.3%), group 4 (20.3%) and non-WNT/non-SHH (18.5%). From 40 patients with clinical information available, 3-year overall survival (n=40) for low, intermediate and high-risk groups were 100%, 60% and 20%, respectively (p<0.05), based only in molecular criteria, which confirmed the prognostic importance of this method. CONCLUSIONS: At an estimated cost of $220 per patient, we are able to implement central molecular diagnosis for the incorporation into a prospective clinical trial protocol in Latin America.

MBRS-67. ROLE OF CYCLIN DEPENDENT KINASE-9 IN *MYC*-ENHANCED MEDULLOBLASTOMA

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Myc is highly expressed in group 3 medulloblastoma (Myc-MB) and influences cell growth, proliferation and oncogenesis by directly promoting the activity of RNA polymerases (RNA Pol). Myc driven RNA Pol II activity is mediated by Positive Transcription Elongation Factor b (pTEFb). pTEFb's catalytic core consists of cyclin dependent kinase-9 (CDK9) and Cyclin T, that phosphorylate and release RNA Pol II into active elongation. CDK9 is over expressed in group 3 MB suggesting that MB may be vulnerable to inhibition of CDK9 (CDK9i). The exact mechanism is not completely known in MB. Genetic depletion of CDK9 suppressed Myc-MB cell clonogenicity *in vitro* and tumor growth *in vivo*. CDK9i by two clinically relevant inhibitors, Atuveciclib and AZD4573, suppressed clonogenicity and cell self-renewal of Myc-MB cell lines. CDK9i in Myc-MB cell lines downregulated Myc and RNA Pol II phosphorylation at Ser2 and Ser5, and, upregulated P21. Further, mice with orthotopic xenografts treated with CDK9 inhibitors survived significantly longer than control mice. RNA-Seq-based gene set enrichment analysis showed that CDK9i decreased c-Myc driven transcriptional programs and enhanced differentiation networks. ChIP-Seq for Pol2 and Myc, demonstrated that the Myc-driven aberrant transcriptional input can be reversed via CDK9i. These findings highlight the role of CDK9 in Myc-driven pathogenesis and that its inhibition is critical to the treatment of Myc-MB.

MBRS-68. SINGLE NUCLEUS RNA-SEQUENCING DECIPHERS INTRATUMORAL HETEROGENEITY IN MEDULLOBLASTOMA WITH EXTENSIVE NODULARITY (MBEN)

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Medulloblastoma (MB) with extensive nodularity (MBEN) represent a rare subtype of cerebellar tumors of infancy which comprise two histologically distinct components, nodular reticulin-free zones and inter-nodular reticulin-rich regions. We applied single nucleus RNA-sequencing (snRNA-seq) using the 10X Genomics and the SMARTseq V2 protocols, bulk RNA-sequencing, DNA-methylation profiling and DNA-panel sequencing to ten histologically confirmed MBEN specimens. All tumors were classified as sonic hedgehog (SHH) MB based on DNA methylation. Somatic mutations within the SHH-pathway were detected in seven samples (3x *SUFU*, 2x *PTCH1*, 2x *SMO*) by DNA panel sequencing. The combined snRNAseq approach resulted in data on ~30.000 single cells. Several non-malignant cell types were identified, e.g. endothelial cells, astrocytes, and microglia. Amongst malignant cell populations SHH-pathway activation and mitotic

activity differed revealing actively cycling embryonic stem (ES) cell-like and more differentiated neuronal-like cell types. In addition, distinct histological components of these tumours were subjected to bulk RNA sequencing following microdissection. This approach was repeated for DNA methylation profiling in an independent paraffin embedded MBEN cohort. However, these analyses did not reveal significant transcriptomic differences or differential methylation patterns between the two histological components. In summary, snRNA-seq identified a strongly proliferating, ES-like subset of cells in MBEN, which might represent the driving cell population in these malignancies, while direct analyses of nodular and inter-nodular regions did not reveal any significant differences. These findings suggest that both components originate from the same cell of origin but represent different cellular developmental stages.

MBRS-69. METABOLITE PROFILING OF SHH MEDULLOBLASTOMA IDENTIFIES A SUBSET OF CHILDHOOD TUMOURS ENRICHED FOR HIGH-RISK MOLECULAR BIOMARKERS AND CLINICAL FEATURES

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SHH medulloblastoma patients have a variable prognosis. Infants (<3–5 years at diagnosis) are associated with a good prognosis, while disease-course in childhood is associated with specific prognostic biomarkers (*MYCN* amplification, *TP53* mutation, LCA histology; all high-risk). There is an unmet need to identify prognostic subgroups of SHH tumours rapidly in the clinical setting, to aid in real-time risk stratification and disease management. Metabolite profiling is a powerful technique for characterising tumours. High resolution magic angle spinning NMR spectroscopy (HR-MAS) can be performed on frozen tissue samples and provides high quality metabolite information. We therefore assessed whether metabolite profiles could identify subsets of SHH tumours with prognostic potential. Metabolite concentrations of 22 SHH tumours were acquired by HR-MAS and analysed using unsupervised hierarchical clustering. Methylation profiling assigned the infant and childhood SHH subtypes, and clinical and molecular features were compared between clusters. Two clusters were observed. A significantly higher concentration of lipids was observed in Cluster 1 (t-test, p=0.012). Cluster 1 consisted entirely of childhood-SHH whilst Cluster 2 included both childhood-SHH and infant-SHH subtypes. Cluster 1 was enriched for high-risk markers - LCA histology (3/7 v. 0/5), *MYCN* amplification (2/7 v. 0/5), *TP53* mutations (3/7 v. 1/5) and metastatic disease - whilst having a lower proportion of *TERT* mutations (0/7 v. 2/5) than Cluster 2. These pilot results suggest that (i) it is possible to identify childhood-SHH patients linked to high-risk clinical and molecular biomarkers using metabolite profiles and (ii) these may be detected non-invasively *in vivo* using magnetic-resonance spectroscopy.

MBRS-70. FUNCTIONAL DEPENDENCY BETWEEN REST AND DNMT1 IN MEDULLOBLASTOMA

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Medulloblastomas exhibit poor neuronal lineage specification. Expression of RE1 Silencing Transcription Factor (*REST*), a repressor of neurogenesis, is aberrantly elevated in human sonic hedgehog (SHH) medulloblastomas. Constitutive REST expression in mice (*RESTTG*) drives medulloblastoma genesis and promotes tumor progression in the context of *Ptch1* haploinsufficiency (*Ptch+/–*), implicating it as a driver of tumorigenesis. Tumor formation in *Ptch+/–/RESTTG* mice showed significantly decreased latency and increased penetrance compared to that in *Ptch+/–* mice. Since REST silences gene expression by chromatin remodeling, we sought to identify cooperating epigenetic events that contributed to its oncogenic