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

activity. To this end, we performed a loss of function screen employing a bar-coded library of short hairpin RNAs against epigenes, to identify candidates whose loss could create a proliferative vulnerability in the context of REST-elevation. This screen identified DNA methyltransferase 1 (DNMT1) as a high-priority epigenetic modifier. DNMT1 and the Ubiquitin like with PHD and Ring finger domain 1 (UHRF1) proteins are essential for methylation of hemi-methylated DNA at the replication fork during S-phase. Their expression is downregulated during neuronal differentiation. In human SHH-medulloblastoma tumors, *REST* and *UHRF1* expression are positively correlated with higher levels of both genes noted specifically in the SHH-beta subtype, and is associated with poor prognosis. The requirement for DNMT1/UHRF1 in the context of REST elevation, was established by RNA-Seq and Reduced Representation Bisulfite Sequencing (RRBS), which revealed hypermethylation and downregulated expression of REST-target genes needed for neurogenesis. Thus, DNMT1/UHRF1 is a functional and potential therapeutic vulnerability in REST-elevated SHH medulloblastomas.

MBRS-71. ATAXIA TELANGIECTASIA AND RAD3-RELATED PROTEIN ATTENUATES DNA DAMAGE AND IS A THERAPEUTIC TARGET IN MYC-DRIVEN MEDULLOBLASTOMA

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Group 3 medulloblastoma tumors (Myc-MB), and particularly the 3γ subtype, have the worst prognosis and show a 5-year overall survival of less than 40%. Group 3 tumors are often accompanied by *Myc* amplification and have a higher rate of metastatic disease and relapse. Unfortunately, therapeutic strategies to target *Myc* have remained elusive. Further, the relapse of the MB has been linked to DNA replication stress. Ataxia telangiectasia and Rad3-related protein (ATR) senses persistent DNA damage, which arises due to replication stress, and activates damage checkpoints, thereby leading to increased cell survival. ATR is highly expressed in MB and is thought to contribute to undisturbed DNA replication to protect genomic integrity. Yet, the exact underlying mechanisms involving ATR are still unclear in MB. Inhibition of ATR (ATRi) using the ATR inhibitor, AZD6738, suppressed clonogenicity and cell self-renewal in Myc-MB. ATRi in Myc-MB cell lines downregulated Chk1 and upregulated P21. ATRi also induced cell cycle arrest and increased apoptosis in Myc-MB cell lines. Further, mice with orthotopic xenografts treated with ATR inhibitor survived significantly longer than control mice. High-throughput drug screening showed ATRi to be synergistic with chemotherapeutic agents including gemcitabine, cisplatin and topotecan. The treatment of Myc-MB cells with ATR inhibitor in combination with gemcitabine and with radiation increased in expression of DNA damage markers. These findings emphasize the role of ATR in alleviating DNA replication stress and that its inhibition is critical to the treatment of Myc-MB.

MBRS-72. MIR-212 FUNCTIONS AS A TUMOR SUPPRESSOR GENE IN GROUP 3 MEDULLOBLASTOMA VIA TARGETING NUCLEAR FACTOR I/B (NFIB)

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Medulloblastoma (MB), the most frequent malignant pediatric brain tumor is divided into four primary subgroups, i.e. wingless-type (WNT), sonic hedgehog (SHH), group 3, and group 4. Haploinsufficiency of chromosome 17p13.3 and c-myc amplification distinguish high-risk group 3 tumors and are associated with rapid recurrence and early mortality. We sought to identify the role of miR-212, which resides on chromosome 17p13.3, in the pathophysiology of group 3 medulloblastoma. RNA expression analyses revealed dramatically reduced levels of miR-212 in group 3 tumors and cell lines mainly through epigenetic silencing via histone modification (deacetylation). Restoring *in vitro* expression reduced tumor cell proliferation with decreased p-AKT and p-ERK levels, colony formation, migration and invasion in group 3 MB. Interestingly, a shift in differential c-myc phosphorylation (from serine-62 to threonine-58) was noted, resulting in reduced total c-myc levels, concurrent with elevated cellular apoptosis. In turn, pro-apoptotic binding partners of c-myc, i.e. Bin-1 and P19[ARF], were upregulated in these cells. A dual luciferase assay confirmed direct targeting of miR-212 to NFIB, a nuclear transcription factor implicated in metastasis and recurrence. Concurrently, increased expression of NFIB was confirmed in group 3 MB tumors with poor survival in high NFIB-expressing patients. Transient NFIB silencing *in vitro* reduced tumor cell proliferation, migration and invasion, and medullosphere formation along with a reduction in

stem cell markers (Nanog, Oct4, Sox2, CD133) and the multi-drug resistance maker, ABCG2. Taken together, these results substantiate the tumor suppressive role of miR-212 in group 3 medulloblastomas and provide a potential new therapeutic target, NFIB.

MBRS-73. AN EXPLORATORY ANALYSIS LOOKING AT THE ASSOCIATION OF GERMLINE CODING MUTATIONS WITH IMPAIRED DEVELOPMENT AND ADAPTIVE BEHAVIOR FUNCTION IN PEDIATRIC MEDULLOBLASTOMA PATIENTS TREATED ON HEAD START 4

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Children with brain tumors often carry germline mutations known to contribute to tumorigenesis and treatment response; however, little is known about how these mutations impact developmental and behavioral outcomes. As the molecular mechanisms governing cancerous and normal tissues expand, we hypothesize that specific germline variants may impact baseline neurocognitive function and/or treatment-induced toxicities. In this pilot study, ten children on the Head Start 4 (HS4) clinical trial diagnosed with medulloblastoma were assessed for baseline adaptive functioning using the Adaptive Behavior Assessment System Third Edition (ABAS-III) and germline whole-exome sequencing was performed. After filtering for high impact variants, Welch's T-tests were used to identify mutations associated with lower ABAS-III General Adaptive Composite (GAC) scores, reflecting developmental and adaptive behavior delays compared with peers their age. We found twenty genes with alterations associated with lower scores with p-values less than 0.05. Genes found to be significant included *LAMC1* (p=0.04) and *KRTAP1-1* (p=0.045), which encode members of the laminin and keratin family respectively and are involved in extracellular matrix adhesion. Mutations in *PITX1*, a known suppressor of *RAS*, were also associated with lower ABAS-III GAC scores (p=0.007). We hypothesize that additional analyses of HS4 patients will reveal alterations in cell-to-cell communication and signal transduction pathways, common molecular perturbations in tumors that would likely impact central nervous system function. Validation studies are essential to improve our understanding of the functional impact of germline variants on both tumor and regular tissue biology, allowing for novel strategies to circumvent these delays.

PRECLINICAL MODELS/EXPERIMENTAL THERAPY/ DRUG DISCOVERY

MODL-01. SAFETY IN CONCOMITANT USE OF MEK AND BRAF INHIBITORS WITH BEVACIZUMAB

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BACKGROUND: MEK and BRAF inhibitors are increasingly common treatments for pediatric nervous system tumors. While effective in blocking Ras/Raf/MEK/ERK pathway activation driving tumor progression, the side effect profile differs from traditional cytotoxic chemotherapies. Little data exists on overlapping toxicities with other targeted agents like bevacizumab despite their potential combined therapeutic benefit. METHODS: A retrospective review of patients treated with MEK +/- BRAF inhibitors and bevacizumab from 2015–2019 was conducted. Data collected included demographics, tumor type, neurofibromatosis status, treatment duration, reason for concurrent treatment, and toxicities. RESULTS: Fifteen patients aged 3–24 years old (median age 14 years) were identified. Diagnoses included five high-grade gliomas, four low-grade gliomas, four benign nerve sheath tumors, and one ependymoma. Nearly half (46.7%) were positive for neurofibromatosis type 1. Three patients were treated with a BRAF + MEK inhibitor and twelve were treated with a MEK inhibitor combined with bevacizumab. Duration of treatment ranged from 16–420 days (median 119 days). Reasons for concomitant therapy included progressive disease with neurologic decline (46.7%), painful benign nerve sheath tumors (26.7%), and visual loss with optic pathway gliomas (26.7%). Toxicities while on concurrent therapy included one episode of grade 1 left ventricular dysfunction, one grade 1 bleeding episode, and one grade 2 wound complication. There were no episodes of hypertension, thrombosis, GI perforations, or cytopenias. CONCLUSIONS: Our preliminary experience suggests bevacizumab in combination with MEK and BRAF inhibitors can be used