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