highest metastatic rate, however G4 γ showed the worst survival. CONCLUSION: We identified molecular subgroups and subtypes of MBs based on gene expression and DNA methylation profile in children in our cohort series. The results may contribute to the establishment of nation-wide correlated optimal diagnosis and treatment strategies for MBs in infant and children.

MBRS-16. MYC REGULATED LONG NONCODING RNA LNC-HLX-2–7 IS A PUTATIVE MOLECULAR MARKER AND A THERAPEUTIC TARGET FOR GROUP 3 MEDULLOBLASTOMAS IN CHILDREN

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Medulloblastoma (MB), a central nervous system tumor that predominantly affects children, requires aggressive therapy. Recent advances in the noncoding RNA genome could contribute to the sub-classification of medulloblastoma. The focus of this study is to identify novel long noncoding RNAs (lncRNAs) as molecular markers and potential therapeutic targets within each subgroup of MBs, in particular within Group 3. We analyzed publicly available 175 RNA-seq datasets to identify a group of putative lncRNA signatures that may be able to differentiate medulloblastoma subgroups accurately. Among those, lncRNA *lnc-HLX-2–7* was highly upregulated in Group 3 MB cell lines, patient-derived xenografts, FFPE samples compared to other groups. CRISPR/Cas9 deletion of the *lnc-HLX-2–7* followed by the fluorescence-activated sorting and generating monoclonal Group 3 MB cells significantly reduced the cell growth and 3-D colony formation together with the induction of apoptosis. Intracranial injection to mouse cerebellum using *lnc-HLX-2–7* deleted cells resulted in reduced tumor growth compared to parental cells, and tumors were further characterized by single-cell sequencing. We identified that oncogene MYC regulates *lnc-HLX-2–7* and its expression can be controlled by the small molecule JQ1, a BET-bromodomain (BRD4) inhibitor that disrupts interactions with MYC. RNA-FISH analysis using FFPE, PDX, and tissue microarrays revealed that *lnc-HLX-2–7* expression is specific to Group 3 MB compared to other groups. We present supporting evidence that *lnc-HLX-2–7* is a novel molecular marker and a potential therapeutic target for Group 3 MBs in children.

MBRS-17. EXAMINING THE ROLE OF LHX9 IN GROUP 3 MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant brain tumor of childhood. Despite major advances in our understanding of the biology of MB, novel treatments remain urgently needed. Using a chemical-genomics driven drug repositioning strategy, we identified the cardiac glycoside family of compounds as potential treatments for Group 3 MB. We subsequently demonstrated that single-agent treatment with digoxin prolongs survival in a patient-derived xenograft model (PDOX) of Group 3 MB to a degree comparable to radiation therapy, a mainstay in the treatment of MB. Finally, we examined the mechanism of digoxin-mediated cell killing using RNA-seq. This work identified LHX9, a member of the LIM homeobox family of transcription factors, as the gene most significantly down-regulated following treatment (Huang and Injac et al, *Sci Trans Medicine*, 2018). Homologs of LHX9 play key roles in cerebellar development via spatially and temporally restricted expression and LHX9 has been proposed as a core transcription factor (TF) in the regulatory circuitry of Group 3 tumors. Loss of function of other core TFs has been shown to impact MB growth. The role of LHX9 in MB, however, has not been previously experimentally evaluated. We now report that knockdown of LHX9 in MB-derived cell lines results in marked growth inhibition raising the possibility that loss of LHX9 plays a major role in digoxin-mediated cell killing and that LHX9 represents a key dependency required for the growth of Group 3 MB. Clinical targeting of core TFs would represent a novel approach to targeting this devastating disease.

MBRS-18. TUMOR SUPPRESSOR P53 DEFINES THE THERAPEUTIC RESPONSES IN TREATMENT OF MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common primary pediatric malignant brain tumor. Current molecular analysis classifies MB into 4 groups, classic (WNT), sonic hedgehog (Shh), group 3, and group 4. Furthermore, atypical p53 signaling is associated with disease progression and confers poor prognosis. This study investigated the correlation of mutational status of p53 and iSO17q with disease progression and metastatic potential. In addition, we used small molecule inhibitors of PI3K (Buparlisib; BKM120) and HDAC (LBH-589) on a p53-mutant MB cell line to find novel therapeutic targets. Efficacy of these drugs were assessed using functional assays (cell proliferation, migration, cell cycle and drug resistance). MB tumors (n=53) were evaluated for GLI-1, GAB-1, NPR, KV1, YAP expression and mutant p53 via immunohistochemistry and correlated to patient outcomes. Results demonstrated that: 1) high expression of GAB-1 and YAP were found in the Shh group, while KV1 expression was present in all subtypes; 2) mutant p53 expression was present in various subsets of MB with no apparent correlation with metastasis or disease progression; 3) patients displaying iSO17q (determined by fluorescence in situ hybridization (FISH) technique) exhibited metastatic disease; 4) LBH-589 and BKM120 caused both time and dose-dependent inhibition of MB cell proliferation and migration; 5) combined treatment of BKM120 and LBH-589 had a synergistic effect; 6) MB cells demonstrated drug-resistance to BKM120. In conclusion, these findings underscore use of Buparlisib and LBH-589 in treatment of MB. Further, the role of mutant p53 in disease progression remains elusive, whereas presence of iSO17q defines metastatic potential.

MBRS-19. SYNERGISM OF HDAC AND PARP INHIBITORS IN MYC-DRIVEN GROUP 3 MEDULLOBLASTOMA CELLS

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Patients with MYC-driven Group 3 medulloblastoma (MB) show particularly poor outcome. It was previously shown that MYC-driven MBs are highly sensitive to class I histone deacetylase inhibition (HDACi). We studied the molecular effects of the class I HDACi entinostat in MYC-driven MB cells to identify potentially synergistic drug combinations, prioritizing drug clinical availability to enable clinical translation. Gene expression profiles of the MYC-amplified group 3 MB cell line HD-MB03 treated with entinostat were analyzed using bioinformatic approaches, identifying 29 altered biomechanisms. Overlay with a translational drug library of n=76 compounds resulted in 44 compounds targeting 9 biomechanisms. Filtering for publications supporting each drug´s role in MYC-driven entities, or functional interaction with HDACs, without publication of this combination in MBs, resulted in 5 compounds (olaparib, idasanutlin, ribociclib, selinexor, vinblastine). Synergism testing identified olaparib as the drug with the strongest synergism. Validation of the combination olaparib and entinostat by p.H2AX and PI staining as well as trypan blue exclusion showed increased double strand breaks (DSBs), increased cell death, loss of viability and cell numbers. Selectivity of MYC-amplified MB cells was shown by comparison to MYC-non amplified cell lines, which showed higher IC50s, and reacted with cell cycle arrest as opposed to cell death to the combination treatment. The role of HDACis in DNA damage repair was confirmed by increased DSBs when entinostat was added to the combination of olaparib with doxorubicin. Our study identified olaparib as a potential combination partner with entinostat for the treatment of MYC-driven Group 3 MB.

MBRS-20. CSF-DERIVED CIRCULATING TUMOR DNA AS A BIOMARKER FOR DISEASE PROGRESSION AND TUMOR EVOLUTION IN MEDULLOBLASTOMA

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BACKGROUND: Cell-free DNA (cfDNA) profiling has been shown to carry utility as a clinically relevant biomarker in a variety of cancers, but studies in pediatric brain tumors, including medulloblastoma, are scarce. We hereby evaluated the actionability of profiling cfDNA from cerebrospinal fluid (CSF) based on a multi-institutional cohort of children with medulloblastoma. METHODS: 103 children aged ≥ 3 years with medulloblastoma harboring chromosomal aneuploidy enrolled on two prospective therapeutic trials were included. cfDNA was extracted from CSF obtained longitudinally, and profiled by low-coverage whole-genome sequencing (lcWGS) for annotating copy-number variants (CNVs). cfDNA-derived CNVs were compared against patient-matched primary tumor-derived CNVs and correlated with outcome. cfDNA profiles at diagnosis and relapse were compared to evaluate tumor evolution. RESULTS: Tumor-derived somatic CNVs were detected in 72% of baseline cfDNA samples, with higher detection rate in samples from patients with metastatic disease than those without (90% versus 50%, chi-square p=0.001). Longitudinal profiling of cfDNA revealed correlation between CNV detectability and clinical course, with detection of tumor-derived CNVs in cfDNA samples predating radiographic progression for ≥ 3 months in 62% of relapsing patients. Presence of cfDNA-derived CNVs in CSF collected during chemotherapy and at the end of therapy was significantly associated with inferior PFS (log-rank p<0.0001 for both time-points). Comparison of CNV profiles from cfDNA at baseline and relapse revealed molecular divergence in a subset of patients. CONCLUSION: These results carry major implications and supports the incorporation of cfDNA profiling in upcoming medulloblastoma protocols for more sensitive and accurate disease monitoring and personalization of treatment.

MBRS-21. CLINICAL AGGRESSIVENESS OF TP53-WILD TYPE SONIC HEDGEHOG MEDULLOBLASTOMA WITH MYCN AMPLIFICATION

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Clinical implication of MYCN amplification in sonic hedgehog (SHH) medulloblastoma may still be controversial due to the frequent co-occurrence with TP53 mutation, which is one of the poorest prognostic factors among the subgroup. We described two cases of TP53-wild type SHH medulloblastoma with MYCN amplification, showing dismal clinical course with rapid disseminated relapse just after the end of treatment. CASE 1: A 7-year-old boy developed a non-metastatic cerebellar tumor. Pathology of the tumor was consistent with classic medulloblastoma. The patient received treatment that involved reduced-dose (18 Gy) craniospinal irradiation (CSI), local irradiation, and chemotherapy. However, sudden respiratory arrest developed due to massive intracranial disseminated relapse 9 months after the initial surgery. CASE 2: A 6-year-old boy presented a large mass in his 4th ventricle without dissemination. He diagnosed with large cell/anaplastic medulloblastoma and underwent radiation therapy (24 Gy of CSI and local irradiation) and chemotherapy, followed by high-dose chemotherapy. However, dissemination through neuroaxis occurred 9 months after the diagnosis. Methylation data of the cases was entered into a recently published classifier and both tumors were classified as "medulloblastoma, subclass SHH A (children and adult)". Copy number analysis demonstrated MYCN amplification in both cases. TP53 mutation analysis from exon 2 to 10 indicated wild type in one case. Additionally, p53 immunochemistry in both cases also indicated wild type. The cases remind us of the clinical aggressiveness of SHH medulloblastoma with MYCN amplification, even if there is no TP53 mutation. The tumor should still be treated with the most intensified treatment.

MBRS-22. SIGNIFICANCE OF *RNF213* IN TUMORGENICITY OF MEDULLOBLASTOMA

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RNF213 gene, initially identified as a disease-causing gene for moyamoya cerebrovascular disease, has recently been recognized as a tumor regulator. The gene is known to be associated with WNT signaling, lipid metabolism, angiogenesis and genomic instability. The purpose of this study was to investigate the association of *RNF213* in tumorgenicity of medulloblastoma. Incidence of medulloblastoma and histopathological findings were compared among *ptch1*+/-, *ptch1*+/- *rnf213*+/-, and *ptch1*+/- rnf213-/- mice. Knockout of *rnf213* in *ptch1*+/- transgenic mouse model increased the incidence of spontaneous generation of medulloblastoma from 19.8% (*ptch1*+/-) to 76.5% (*rnf213*+/- *ptch1*+/-) at 9 months ($p < 0.001$). Heterozygous knockout was equivalent to homozygous knockout. Haploinsufficiency of *rnf213* seems to be associated with tumorgenicity in medulloblastoma. Molecular mechanism of medulloblastoma generation needs to be further investigated.

MBRS-23. SIGNIFICANCE OF MI-R33 IN GENERATION AND PROGRESSION OF MEDULLOBLASTOMA

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Lipid metabolism has been shown to be associated with tumorigenicity in various malignancies. The purpose of this study was to investigate the association of miR-33, a key regulator of lipid metabolism, in tumorigenicity and progression of medulloblastoma. miR-33a is an only isotype of miR-33 in rodents although miR-33b is also detected in human. Incidence of medulloblastoma and histopathological findings were compared between ptch1+/- mice and ptch1+/- miR-33a-/- mice. Effect of miR-33b upregulation by cordycepin was tested in DAOY medulloblastoma cells both in vitro and in vivo. Knockout of miR-33a in ptch1+/- transgenic mouse model increased the incidence of spontaneous generation of medulloblastoma from 19.8% to 49.5% ($p < 0.001$) at 10 months. Cordycepin, which upregulates miR-33b, prevented tumor growth in DAOY human medulloblastoma cell line, but the effect was not evident in an orthotopic mouse medulloblastoma model. Although miR-33 seems to be an important regulator of medulloblastoma, treatment efficacy of cordycepin was not enough. Combination treatment with immunotherapy or cytotoxic treatment needs to be tested to show survival benefit in preclinical models.

MBRS-24. FUNCTIONAL CHARACTERIZATION OF IKBKAP/ELP1 AS A NOVEL SHH MEDULLOBLASTOMA PREDISPOSITION GENE

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Medulloblastoma (MB), a common malignant pediatric brain tumor, comprises at least four distinct molecular entities: WNT, SHH, Group 3, and Group 4. SHH-MB is driven by aberrant activation of the Sonic hedgehog (SHH) pathway in granule neuron progenitors (GNPs) and is associated with hereditary cancer predisposition syndromes including Li Fraumeni and Gorlin. We recently identified germline loss of function (LoF) mutations affecting *IKBKAP/ELP1*, the primary scaffolding subunit of the Elongator complex in a subset of SHH-MB patients. Germline *ELP1* mutations account for ~15% of all pediatric SHH-MBs and position *ELP1* as the most prevalent hereditary predisposition gene in MB. We genetically engineered *Elp1* LoF in mouse GNPs to determine Elp1 function in cerebellar development and SHH-MB. Results of both mechanistic and phenotypic experiments demonstrate that GNPs harboring *Elp1* loss exhibit ribosome pausing and protein aggregation, reinforcing the critical role of Elp1 in translational elongation and protein homeostasis. Further, we generated new transgenic mouse models mimicking germline *ELP1* LoF mutations observed in SHH-MB patients. *Elp1*+/- transgenic mice exhibit purkinje cell