

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, Atvececlib 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 transcriptomic 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 reticulon-free zones and inter-nodular reticulon-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 (*Ptch1*^{-/-}), implicating it as a driver of tumorigenesis. Tumor formation in *Ptch1*^{-/-}/*RESTTG* mice showed significantly decreased latency and increased penetrance compared to that in *Ptch1*^{-/-} mice. Since *REST* silences gene expression by chromatin remodeling, we sought to identify cooperating epigenetic events that contributed to its oncogenic