SIONS: We found that pre-treatment NLR was an independent prognostic factor for recurrence or metastasis of medulloblastoma after treatment. In combination with NRL and clinical factors, nomogram has a good prediction of PFS in patients with medulloblastoma after radiotherapy. It has the potential to facilitate more precise risk stratification to guide personalized treatment of medulloblastoma.

MEDB-59. A DRAFT ATLAS OF MEDULLOBLASTOMA CELLULAR EVOLUTION UNDER THERAPY

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How standard care shapes the cellular composition of recurrent medulloblastoma (MB), if therapy selects for specific tumor or immune cell types, is unknown. We report the pilot phase of our ongoing effort to profile human longitudinal MB specimens via single-cell transcriptomics and epigenetics. We profiled 11 diagnostic and eight recurrent specimens from 19 subjects via single-nucleus RNA sequencing (snRNA-seq), and four subjects via single-nucleus assay for transposase-accessible chromatin. Specimens from select subjects were also profiled to assess genome-wide enhancer activity via single-nucleus cleavage-under-targets and tagmentation. We found an upregulation of the DNA-damage response, RNA translation, WNT and NOTCH signaling in recurrent specimens. The percentages of stem-like cells increased by over two-fold at recurrence. We found that microglia and oligodendrocyte-lineage cells were the most abundant non-malignant tumor-associated cell types, representing 2%-10% of cells profiled. Microglia abundances were relatively stable across molecular subtypes, and when comparing primary to recurrent tumors. There was a moderate, but statistically significant, increase in oligodendrocyte abundance in SSH and WNT tumors, compared to Group 3/4 tumors. We compared gene expression in tumor cells with public snRNA-seq from developing human cerebella (PCW 9-21). Combined Group-3/4 cell analysis supports a common lineage hierarchy, with an enrichment for unipolar brush-cell and Purkinje-cell phenotypes found in Group-4 tumors. All Group-3/4 cases contained cycling cells expressing markers of PAX2+ interneuron progenitors, most cycling cells had this phenotype. All specimens contained populations of non-cycling granule-cell progenitor-like cells. We performed single-cell co-expression receptor/ligand analysis to infer paracrine signaling between tumor and non-malignant cell types. This identified both tumor cells and microglia as sources of growth factors, pro-inflammatory cytokines, and pro-apoptotic ligands. Non-malignant oligodendrocyte-lineage cells uniquely expressed IL6-family cytokines, pleiotrophin, and class-III semaphorins. These studies shed light on the cellular heterogeneity of MB and the effect of standard therapy in shaping composition at recurrence.

MEDB-60. MEDULLOBLASTOMA WITH EXTENSIVE NODULARITY MIMICS CEREBELLAR DEVELOPMENT AND DIFFERENTIATES ALONG THE GRANULAR PRECURSOR LINEAGE

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BACKGROUND: Medulloblastoma with extensive nodularity (MBEN) represents a rare type of cerebellar tumors of infancy comprising two histologically distinct components that differ in cell differentiation and mitotic activity. Whereas some children suffering from MBEN experience disease recurrence, MBEN can also spontaneously differentiate and discontinue to grow. The underlying mechanisms of this variable biological behavior may offer insight into how embryonal tumors develop. METHODS: Fresh frozen and FFPE tumor tissue from nine MBEN-patients was subjected to multi-omics characterization including bulk sequencing, microdissection followed by RNA sequencing, single nucleus RNA-sequencing using the 10X Genomics- and SMART Seq. V2-protocols and spatial transcriptomics via RNAscope. RESULTS: All cases were molecularly classified as Sonic Hedgehog (SHH)-MB, and harbored somatic mutations within the SHH-pathway. After quality control, a total of ~30.000 cells were subjected to downstream analysis. Several non-malignant cell types, such as glial cells, were identified. In accordance with previous studies, we found only sparse immune infiltration. Unsupervised clustering identified cell clusters that differed in differentiation state and represented a continuum from embryonal-like cells with SHH-upregulation over intermediate cell states, to neuronal-like, postmitotic cells. Mapping to a single nucleus sequencing atlas of cerebellar development indicated that tumor cells reflected various stages of normally developing cerebellar granular precursors. Interestingly, one cluster of malignant cells with tumor-specific copy number alterations showed both transcriptomic features of astrocytes and embryonal cells. Using spatial transcriptomics, we were able to correlate different clusters of MBEN cells with distinct histologic MBEN compartments, with astrocyte-like tumor cells being located in the internodular compartment and in close proximity to mitotically active cancer cells. CONCLUSION: MBEN is formed by a continuum of malignant cell differentiation along the granular precursor lineage, with a subset of cells developing into cells that may represent tumor astrocytes. This differentiation process is reflected in the bicompartmental structure of MBEN.

MEDB-61. GENETIC ALTERATIONS OF *TP53* AND *OTX2* INDICATE INCREASED RISK OF RELAPSE IN WNT MEDULLOBLASTOMAS

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PURPOSE: This genetic analysis of WNT-activated medulloblastomas (WNT-MBs) aimed to re-evaluate the prognostic impact of *TP53* mutations and to identify specific chromosomal aberrations as possible prognostic markers in a retrospective cohort of patients treated according to the protocols of the HIT medulloblastoma studies. PATIENTS AND METHODS: In a cohort of 191 patients with WNT-MBs, mutations in *CTNNB1*, *APC*, and *TP53* were analyzed by Sanger and/or NGS panel sequencing. Chromosomal copy number aberrations (CNAs) were assessed by high-resolution, genome-wide molecular inversion probe technology (MIP), SNP6 array, and/or 850k methylation bead-array hybridization. Complete clinical data were available from 120 patients. RESULTS: Patients with WNT-MBs had a female predominance (1.4:1) and a median age of 13 years (range 3-69 years). *CTNNB1* mutations were present in 92.2% of the samples, *APC* mutations in 6.8%. One *CTNNB1* wildtype tumor gained WNT-activation due to a homozygous deletion of *FBXW7*. Monosomy 6 was present in 78.6%, but more frequent in children compared to adults. 16.1% of the tumor samples showed *TP53* mutations, of those 60% with nuclear positivity for the p53 protein. A loss of heterozygosity at the *TP53* locus on chromosome 17p13.1 was found in 40.7% (11/27) of *TP53* mutant tumor samples and in 18.5% of the whole cohort (24/130 cases). Patients with tumors harboring *TP53* mutations showed significant worse progression-free survival (PFS; p=0.001), but not overall survival (OS) and were enriched for chromosomes 17p (p=0.001), 10, and 13 losses. Gains of the *OTX2* locus on chromosome 14q were also associated with poor PFS and OS (p=0.017 resp. p=0.006). Multivariate Cox regression analysis identified both genetic alterations as independent prognostic markers for PFS and OS. CONCLUSION: For ongoing and future de-escalation trials for patients with WNT medulloblastomas, we recommend to exclude patients with tumors carrying *TP53* mutations or *OTX2* gains.

MEDB-62. DISEASE-ASSOCIATED KBTBD4 MUTATIONS IN MEDULLOBLASTOMA ELICIT NEOMORPHIC UBIQUITYLATION ACTIVITY TO PROMOTE COREST DEGRADATION

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Genomic studies in medulloblastoma have identified distinct disease subgroups: wnt/wingless (WNT), sonic hedgehog (SHH), and non-WNT/non-SHH, comprising group 3 and group 4. Alterations in WNT and SHH signalling form the pathogenetic basis for their subgroups, whereas those for non-WNT/non-SHH tumours remain largely elusive. Recent analyses have revealed recurrent in-frame insertions in the E3 ubiquitin ligase adaptor Kelch Repeat and BTB Domain Containing