MEDB-41. Identifying a subgroup of patients with early childhood sonic hedgehog-activated medulloblastoma with unfavorable prognosis after treatment with radiation-sparing regimens including intraventricular methotrexate [Abstract]

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BACKGROUND: Follow-up examinations are an essential part of the aftercare of patients with brain tumours. We investigated survival in relation to neurological impairment and positive CSF findings at first relapse/progression of medulloblastomas. METHODS: We collected data from patients with relapsed medulloblastoma from the German HIT-REZ studies (HIT-REZ-1997, HIT-REZ-2005, HIT-REZ-Register, n=342). Survival differences dependent on tumour cell-positive and -negative CSF cytology as well as on new onset or worsening of neurological impairment (i.e. headache, nausea/vomiting, ataxia, seizures and others) were analysed. RESULTS: 247 patients with a recurrent medulloblastoma were evaluable for CSF cytology at first relapse/progression (positive n=97, negative n=150). Patients with tumour cell-positive CSF results showed a significantly shorter median PFS and OS time compared to patients with negative CSF cytology [PFS: 9.1 (CI: 5.3-12.9) vs. 16.8 (CI: 13.8-19.8) months, plog rank test=0.001; OS: 14.4 (CI: 12.3-16.4) vs. 41.8 (CI: 33.3–50.4) months, plog rank test<0.001]. The shortest PFS and OS were observed in SHH-activated (n=18) and group 3 medulloblastomas (n=23) independently of CSF cytology result [median PFSSHH: 4.3 (CI:1.1-12.2), OSSHH: 6.3 (CI:1.1-18.7); PFSgroup3: 4.2 (CI:2.3-13.1), OSgroup3: 13.2 (CI:7.1-18.5) months]. For analysis of the impact of neurological deterioration on survival at first relapse, 249 Patients were evaluable. 105 patients with new or severely worsened neurological impairment at first relapse/progression displayed a significantly poorer PFS and OS time in comparison to 144 patients with unchanged or improved neurological symptoms [PFS: 8.2 (CI: 6.0-10.3) vs. 14.9 (CI: 12.0-17.9) months, plog rank test=0.001; OS: 15.1 (CI: 9.5-20.6) vs. 32.6 (CI: 26.2-38.4) months, plog rank test<0.001]. CONCLUSIONS: Patients with relapsed medulloblastoma show significantly worse survival (PFS and OS) in presence of positive CSF cytology or neurologic deterioration at relapse. These findings could be relevant for patient/parents counselling and treatment recommendations at relapse. Funded by the German Children Cancer Foundation

MEDB-39. ONCOGENIC MECHANISMS UNDERLYING GLI2-AMPLIFIED MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant brain tumor in children. There are several subtypes of MB, and among them, the subtype of GLI2-amplified SHH-MB associated with P53 mutations has the worst prognosis and a poor survival rate; the 5-year survival rate is <30%. Moreover, the GLI2-amplified MBs are non-responsive to the only targeted treatment option available for SHH-MB, the SMO inhibitors. This leaves an unmet critical treatment gap, and there is an urgent need to identify novel targets to develop effective therapeutics. However, a deeper understanding of the cellular and molecular mechanisms driving GLI2-amplified MB tumorigenesis is currently lacking. With a focused goal to resolve this particular type of MB tumorigenesis, we recently generated an engineered mouse model of GLI2-driven MB. Using this model, we demonstrated that GLI2 is the critical driver of tumorigenesis and identified granule cell progenitors (GCPs) as the cells of origin. Interestingly, we have also found that GLI2 drives only Math1+ embryonic GCPs but not neonatal GCPs to form SHH-MB. Correspondingly, our scRNA-seq analysis revealed that the MAPK pathway is specifically enriched in embryonic but not neonatal Math1+ GCPs. Moreover, the MAPK pathway is activated in mouse and human GLI2-driven MB tumors, and a MEK/ERK inhibitor significantly delayed the growth of GLI2-driven MB in vivo. Based on these exciting results, we hypothesize that GLI2-driven MB originates from a specific cell population of Math1+ GCPs and in a particular spatiotemporal window during cerebellar development, and targeting MAPK/MEK/ERK pathway may represent a novel effective approach to treating GLI2-amplified MB.

MEDB-40. RUNNING FOR INCLUSION IN SIOPE PNET5 MB

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Enrolling medulloblastoma(MB) patients in the PNET5 protocol is a daily problem in Italy; since June 2015, 59 cases have been enrolled in 13 centres. So far, 44 of the 103 patients claiming for eligibility did not enter the protocol: 13 metastases, 5 for residual, 20 having exclusion criteria, 4 insufficient frozen material, 2 failure to comply with the correct procedures. No case was lost due to delayed centralization, which is respected even with committing weekends; review of the radiation plan was performed on Saturday for 2 cases, and radiotherapy began on the same day. We made some procedural changes to meet expected deadlines; each local centre notifies the national coordinator of a possible case's existence at MRI diagnosis, of the expected surgery date as well as its realization. MRI imaging is reviewed within 2 days after centralization. Paediatricians notify the national coordinator and pathology/biology reference centre of the MB diagnosis; the shipment of frozen tissue, blood and FFPE is booked. A slot is reserved to priority perform the central pathology review, as well as central molecular diagnosis of genetically defined subgroup (WHO classification) upon receipt of the frozen material. Upon receipt of the FFPE and frozen material, the national reference centre undertakes a double-check with the national coordinator and the local treatment centre to validate the eligibility. Within the 7th day from the receipt of the material: IHC, MYC/MYCN, Monosomy 6, beta-catenin mutation and methylation array are performed. Priority execution of somatic (blood control) sequencing of the PTCH, SUFU, and TP53 genes is also triggered for SHH-activated MB, with the deadline on the 15th day. So far we have had 99% agreement between molecular subgrouping and methylation array. CONCLUSIONS: PNET5 requirements are multiple and changing over time; difficulties may and must be overcome by mutual fast collaboration.

MEDB-41. IDENTIFYING A SUBGROUP OF PATIENTS WITH EARLY CHILDHOOD SONIC HEDGEHOG-ACTIVATED MEDULLOBLASTOMA WITH UNFAVORABLE PROGNOSIS AFTER TREATMENT WITH RADIATION-SPARING REGIMENS INCLUDING INTRAVENTRICULAR METHOTREXATE

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PURPOSE/METHODS: Clinical and molecular risk factors in 142 patients <5 years with desmoplastic medulloblastoma (DMB) or medulloblastoma with extensive nodularity (MBEN) were investigated. Patients were diagnosed between 1992 and 2020 and treated with radiation-sparing approaches, 131 with intraventricular methotrexate. 14 patients with metastatic disease received high-dose chemotherapy. DNA methylation profiles of 77 sonic hedgehog (SHH)-activated medulloblastoma were reclassified according to the Heidelberg Brain Tumor Classifier Version 12.3. RESULTS: While metastatic disease or incomplete resection did not impact progression-free survival (PFS) and overall survival (OS), patients with MBEN had superior outcomes to DMB (5-year PFS 93% vs 71%, p=0.004; 5-year OS 100% vs 90%, p=0.026). Older patients had less favorable PFS (5-year PFS [>3 years] 47% vs 85% [<1 year] vs 84% [1-3 years], p<0.001). No TP53 mutations were detected (n=47). DNA methylation classification identified three subgroups: SHH-$1_{v12.3}$ (n=39), SHH-$2_{v12.3}$ (n=19), and SHH-$3_{v12.3}$ (n=19), with distinct cytogenetic profiles (chromosome 2 gains in SHH-$1_{v12.3}$, very few alterations in SHH-$2_{v12.3}$, and chromosome 9q losses in SHH-$3_{v12.3}$), age profiles (median age [years] SHH-$1_{v12.3}$: 1.7, SHH-$2_{v12.3}$: 0.9, SHH-$3_{v12.3}$: 3.0, p<0.001), and histological distribution (SHH-$2_{v12.3}$: 74% MBEN, SHH-$1_{v12.3}$/SHH-$3_{v12.3}$: 77%/79% DMB, p<0.001). PFS was more unfavorable in patients with SHH-$3_{v12.3}$-medulloblastoma (5-year PFS 53% vs 86% [SHH-$1_{v12.3}$] vs 95% [SHH-$2_{v12.3}$], p=0.002), which remained the only risk factor on multivariable Cox regression for PFS. OS was comparable (5-year OS 94% [SHH-$3_{v12.3}$] vs 97% [SHH-$1_{v12.3}$] vs 100% [SHH-$2_{v12.3}$], p=0.6). 8/9 patients with SHH-$3_{v12.3}$-medulloblastoma received radiotherapy at relapse (6 craniospinal, 2 local [1 Gorlin syndrome, 1 BRCA2 germline mutation], 1 no radiotherapy [Gorlin syndrome]). CONCLUSION: We identify patients with an increased risk of relapse when treated with radiation-sparing approaches among children with early childhood SHH-medulloblastoma. If these tumors differ from SHH-3-medulloblastoma typically described in older children remains to be verified. Treatment recommendations need to consider cancer predisposition syndromes.

MEDB-42. GERMLINE *ELP1* DEFICIENCY PROMOTES GENOMIC INSTABILITY AND SURVIVAL OF GRANULE NEURON PROGENITORS PRIMED FOR SHH MEDULLOBLASTOMA PATHOGENESIS

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Germline loss-of-function (LOF) mutations in *Elongator complex protein 1 (ELP1)* are found in 15-20% of childhood SHH medulloblastoma (MB) and are exceedingly rare in non-SHH-MB or other cancers. *ELP1* germline carriers that develop SHH-MB harbor frequent somatic *PTCH1* mutations and universally sustain loss-of-heterozygosity of the remaining *ELP1* allele through chromosome 9q deletion. ELP1 functions as a scaffolding subunit of the Elongator complex that is required for posttranscriptional modification of tRNAs and maintenance of efficient translational elongation and protein homeostasis. However, the molecular, biochemical, and cellular mechanisms by which ELP1/Elongator LOF contribute to SHH-MB tumorigenesis remain largely unknown. Herein, we report that mice harboring germline *Elp1* monoallelic loss (i.e., *Elp1*+/-) exhibit hallmark features of malignant predisposition in developing cerebellar granule neuron progenitors (GNPs), the lineage-of-origin for SHH-MB. *Elp1*+/- GNPs are characterized by increased replication stress-induced DNA damage, upregulation of the homologous recombination repair pathway, aberrant cell cycle, and attenuation of p53-dependent apoptosis. CRISPR/Cas9-mediated *Elp1* and *Ptch1* gene targeting in mouse GNPs reproduces highly penetrant SHH-MB tumors recapitulating the molecular and phenotypic features of patient tumors. Reactivation of the p53 pathway through MDM2 and PAK4 inhibitors promotes selective cell death in patient-derived xenograft tumors (PDX) harboring deleterious *ELP1* mutations. Together, our findings reveal that germline *Elp1* deficiency heightens genomic instability and survival in GNPs, providing a mechanistic model for the subgroup-restricted pattern of predisposition and malignancy associated with pathogenic *ELP1* germline carriers. These results provide rationale for further preclinical studies evaluating drugs that overcome p53 pathway inhibition in *ELP1*-associated SHH-MB and a renewed outlook for improving treatment options for affected children and their families.*, # Contributed equally

MEDB-43. DEVELOPMENT OF A BIOINFORMATICS PIPELINE FOR IDENTIFICATION OF DIFFERENTIAL DNA METHYLATION EVENTS ASSOCIATED WITH MEDULLOBLASTOMA RELAPSE

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Relapsed medulloblastoma (rMB) is treatment-resistant and fatal in ~95% of cases. The epigenetic features of rMB, and any role as drivers of disease relapse/treatment-resistance have yet to be investigated. We therefore developed a pipeline to identify differentially methylated CpGs (DM-CpGs) and regions (DMRs) in a paired-rMB cohort. Our paired-rMB cohort (n=61, relapsed tumours matched with diagnosis counterparts) with available Illumina Methylation 450K/850K microarray data was processed in *R-Studio*. The packages *Limma* and *DMRcate* were used to perform a paired differential methylation analysis on a filtered selection of array probes (n=335,767), identifying DM-CpGs and DMRs with a 5% FDR. DMRs were further retained if they had a maximum-$\Delta\beta$ of >0.2 and correlated with locus-specific gene expression in a separate paired DNA-methylation array/RNA-seq cohort from medulloblastoma diagnosis samples (n=202). Finally, we created univariable Cox models to assess the prognostic potential of DM-CpGs/DMRs in an independent survival cohort of medulloblastoma diagnosis samples (n=498). Across the paired-rMB cohort, there were few significant differential methylation events initially identified at relapse (n=258 DM-CpGs, n=32 DMRs). Upon sub-analysis by molecular group, MB_{Group4} (n=18 pairs) alone yielded significant findings (n=189 DM-CpGs, n=26 DMRs). Most changes involved hypermethylation events detected at relapse. Multiple DM-CpGs identified at relapse were prognostic for both overall and event-free survival when assessed in our independent cohort (n=22 whole cohort, n=13 Group 4, BH-adjusted p<0.05). When applying the DMR filters, only the MB_{Group4} DMRs passed the $\Delta\beta$ filter (n=18/26), with few correlating with gene expression (n=2, p<0.001), and none demonstrating prognostic significance. This pipeline facilitates exploration of the clinical relevance of epigenome-wide changes in a paired-rMB cohort. We highlight the potential prognostic significance of DM-CpGs, and future work will explore the potential functional role of candidate-genes associated with our DMRs, as novel drivers of rMB.

MEDB-44. TRANSCRIPTOMIC RESOLUTION OF SUBGROUP-SPECIFIC MEDULLOBLASTOMA ARCHITECTURE

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Despite a growing understanding and stratification of medulloblastoma, it remains an aggressive childhood brain tumor with high morbidity and mortality. Multimodal genomic and epigenomic analysis has permitted the classification of medulloblastoma into four subgroups with varying biology and clinical behavior: WNT, Sonic-Hedgehog (SHH), Group 3, and Group 4. In our previously published work, Single-cell RNA sequencing (scRNAseq) identified distinct tumor cell subpopulations in specific medulloblastoma groups. However, this technology is limited by its lack of architectural information. Spatial transcriptomics is a relatively new technology that permits the analysis of gene expression as it occurs within organized tissue. In our ongoing study, we utilized Visium spatial transcriptomics, integrated with scRNAseq data and immunohistochemistry, to analyze frozen samples of medulloblastomas (SHH, Group 4, and Group 3 with and without MYC amplification). In SHH in particular, we were able to identify scRNAseq