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AIMS: We report a cohort of YCMB cases homogeneously treated with HDCT in two Italian institutions, and the prognostic impact of histology and genetics retrospectively evaluated. METHODS: All YCMB (aged≤3 years) treated with upfront HDCT in the period 1998-2019 were included, reclassified according to the WHO2021 classification of CNS tumours. Mutational status ofPTCH1, SUFU, and TP53 was analysed in selected cases. Histology and genetics were correlated with survival, secondary tumours(STs), and cancer predisposition syndromes(CPSs). RESULTS: Fifty-three patients were enrolled (62.3% male), median age 2.2 years. 21 had classic(CMB), 15 desmoplastic/nodular(DMB), 11 MBEN and 6 large-cell/anaplastic(AMB/LCMB) medulloblastoma. Metastases were present in 18. Genomic pattern showed SHH-TP53wt in 29 cases, non-WNT/non-SHH in 22; 2 were SHH-TP53mut. Induction chemotherapy (VCR/HDMTX, HDVP16, VCR/HDCTX and HDCARBO) was followed by 2-3 HDCT courses; irradiation reserved to cases with metastatic disease and/or residual tumours. 22 patients never received irradiation. SHH-TP53wt cases had significantly less metastasis (p=0.002), while non-WNT/non-SHH received more often irradiation (p<0.0001). OS at 5, 10, and 20 yrs was 0.73, 0.70 and 0.57 respectively in the entire cohort; stable at 0.85 (at 5, 10, and 20 yrs) in SHH-TP53wt patients while 0.58, 0.51 and 0.17 in the non-WNT/non-SHH. PFS at 5, 10, 20 yrs was stable at 0.89 in SHH-TP53wt and remained 0.35 in non-WNT/non-SHH. 13/53 patients presented Gorlin Syndrome; 1 had familial MB. 16 STs were reported in 14 cases; life-threatening, irradiation-related STs mainly in non-WNT/non-SHH cases. In SHH-TP53wt benign tumours or related to CPS were reported. **CONCLUSIONS**: This is one of the first series of YCMB treated with HDCT without stratification for stage and histology. The long follow-up highlights the frequency/types of associated CPS and STs; the latter, in non-WNT/non-SHH, were treatment-related and life-threatening.

MEDB-36. CLINICAL AND MOLECULAR HETEROGENEITY WITHIN *MYC* AND *MYCN* AMPLIFIED MEDULLOBLASTOMA

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MYC and *MYCN* are the most commonly amplified oncogenes in medulloblastoma. Their overall association with a poor prognosis has supported their adoption as high-risk disease biomarkers in trials. However, emerging evidence suggests that certain patients with *MYN*/*MYCN* focally-amplified tumours can achieve long-term survival and therefore may suffer unnecessary late-effects associated with intensified therapies. To investigate this heterogeneity, we characterised the molecular and clinico-pathological features of curated cohorts of *MYC* (n=64) and *MYCN* (n=95) amplified tumours, drawn from >1000 diagnostic cases, and assessed their associations with disease outcome. Within the *MYCN*-amplified cohort, survival was related to molecular group; patients with $MYCN_{Grp3}$ or $MYCN_{Grp4}$ tumours with no other clinico-pathological risk factors (subtotal resection (STR), metastatic disease, LCA pathology) were intermediate-risk (n=25;70% 5-year PFS). In contrast, a very-high-risk group was defined by positivity for $MYCN_{SHH}$, STR and/or LCA (n=64;32% 5-year PFS). 22/35 assessable $MYCN_{SHH}$ harboured *TP53* mutations; 9/12 with data were germline. MYC_{Grp3} represented the majority (46/58; 79%) of molecularly-grouped *MYC*-amplified tumours. Importantly, while radiotherapy receipt conferred a modest survival advantage, for *MYC*-amplified tumours with additional clinico-molecular risk factors (LCA, metastasis, STR, Grp3), survival was dismal, irrespective of radiotherapy receipt. A very-high-risk group of *MYC*-amplified tumours was identified (n=51;10% 5-year PFS), defined by positivity for ≥1 additional risk factors (STR, LCA and/or metastasis). Alternatively, membership of subgroups II/V defined a smaller, very-high-risk patient group (n=28;7% 5-year PFS). Long-term survival was seen in the majority of remaining *MYC*-amplified tumours negative for these specified features (61% 5-year PFS; high-risk). *MYC* and *MYCN*-amplified medulloblastomas are biologically heterogeneous with diverse clinical outcomes. Molecular subgroup assignment and established clinical features are critical for their improved stratification. Patient subgroups identified may be eligible for therapy de-escalation; in contrast, the very-high-risk patient groups are incurable using current therapies and urgently require novel experimental treatment strategies upfront.

MEDB-37. CHEMOTHERAPY RESPONSE PREDICTION BY MOLECULAR RISK FACTORS IN METASTATIC CHILDHOOD MEDULLOBLASTOMA

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BACKGROUND: Childhood metastatic medulloblastoma (MB) frequently receive postoperative chemotherapy (CT) before craniospinal irradiation. Some MB show stable (SD) or progressive disease (PD) upon CT. Identification of biomarkers for non-response might allow therapy-modifications. METHODS: Patients registered to the German HIT-MED database (2001–2019) were eligible if they were 4-21 years old at diagnosis of a M2/M3-metastasized MB, received therapy in analogy to the MET-HIT2000-AB4 protocol, had centrally reviewed response assessment after 2 cycles HIT-SKK-CT and DNA-methylation analysis was available. DNA-methylation-based tumor classification and whole chromosomal (WC) losses/gains were derived from DNA-methylation arrays. RESULTS: 51/163 (31.3%) patients (median age: 9.8±4.4 years, median follow-up: 6.2±4.0 years) presented SD/PD during/after HIT-SKK-CT and were classified as non-responder. Response to CT had high predictive value for PFS/OS (5-year PFS responder: 67.9±4.8 %, non-responder: 26.1±6.6%, p<0.01 / 5-year OS responder: 80.0±4.2%, non-responder: 45.9±8.0%, p<0.01). Patients with nonWNT/nonSHH-MB subtype II (response: 7/13), subtype III (response: 6/19) and/or MYC-amplification (n=27, overlap subtype II/III: n=11/8, response: 14/27) were less likely to respond, while all 6 of WNT, 8/9 SHH-TP53-wildtype and 1/1 SHH-TP53-mutant responded (Mann-Whitney-U-test p=0.04). Further, ≥2 WC losses/gains of chromosome 7/8/11 was associated with superior response (n=29/32, others: n=83/131, Mann-Whitney-U-test p<0.01). We identified a very-high-risk-cohort (any two criteria of: <2 WC losses/gains of chromosome 7/8/11, MYC-amplification, MB subtype II, III, V, or VIII, n=94), and a standard-risk-cohort (WNT or any ≥2 WC losses/gains of chromosome 7/8/11, n=37) with 40 vs. 8 % non-response and 44±5/60±5 vs. 79±7/87±6% 5-year PFS/OS (p<0.01/p<0.01), respectively. Non-response in n=32 non-VHR/non-SR-patients was 32% with a 5-years PFS/OS of 60±10/77±8%. CONCLUSION: Molecular information can be helpful to predict response to chemotherapy. Upon validation, this may contribute to improve treatment stratification in metastatic MB.

MEDB-38. SIGNIFICANCE OF CSF CYTOLOGY AND NEUROLOGIC DETERIORATION IN RELAPSED MEDULLOBLASTOMAS IN THE GERMAN HIT-REZ-97/-2005 STUDIES AND THE HIT-REZ-REGISTER

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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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