tent of resection (EOR) and its impact on survival, with particular consideration of EOR in relation to the four MB consensus molecular subgroups (WNT, SHH, Group 3, Group 4). We collected data from 1113 patients (n=419, UK CCLG institutions; n=694, published data) representing the largest ever combined cohort constructed to assess the impact of EOR in medulloblastoma. We performed association analyses and univariate/multivariate survival analysis using Kaplan-Meier, log-rank and Cox proportional hazard modelling, analysing overall survival (OS) cohort-wide and with reference to molecular subgroups and clinical features. Association analysis of the combined cohort evidenced that infant patients were more likely to have STR (p=0.02). In this whole-cohort analysis, EOR was significantly associated with survival in univariate analysis (HR 1.64, 95% CI 1.30-2.07, p=<0.001) but not in multivariate analysis. STR was variably prognostic in sub-cohort analyses of specific demographics and molecular subgroup; worse outcomes were observed in patients <5 years in SHH (p=0.044) and Group 4 (p=0.044). This was true for WNT patients >5 years old at diagnosis (p=0.034) although numbers were small and require validation in even larger cohorts. In this cohort of >1100 MBs, STR was significantly associated with a lower OS in univariate analysis, but this was driven by specific disease contexts (SHH and Group 4 patients <5 years old). STR was not independently prognostic overall or in any setting. We recommended that surgeons should continue to pursue maximal *safe* resection for all MB patients but suggest that consideration of STR as a high-risk feature should be disease context specific.

MEDB-32. REDUCING TREATMENT-RELATED TOXICITY FOR CHILDREN WITH WNT-ACTIVATED MEDULLOBLASTOMA

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WNT-medulloblastoma has an excellent prognosis, with an overall survival rate of 90% among children receiving standard-of-care (SOC) surgical resection, radiotherapy, and chemotherapy. Unfortunately, while curative, this treatment is associated with major, long-term, debilitating motor, developmental, and neuroendocrine side effects. Therefore, it is crucial we develop effective, less toxic therapies for these children. Similarities have been demonstrated between cancer cell lysosomes and those of patients with Niemann-Pick, a lysosomal storage disease characterised by lysosomal fragility and sphingomyelin accumulation. A class of drugs known as Functional Inhibitors of Acid Sphingomyelinase (FIASMAs), increase lysosomal sphingomyelin and destabilise the cancer cell's more fragile lysosomal membrane which leads to the induction of cell-death pathways via lysosomal membrane permeabilisation. Loratadine, an antihistamine with high FIASMA activity, consistently induced lysosomal membrane permeabilisation, leading to increased cell-death, in our panel of mouse and human WNT-medulloblastoma lines. Loratadine exhibited no detrimental effect on normal mouse embryonic stem cells from the lower rhombic lip – the putative cell of origin in WNT-medulloblastoma. Luciferase-expressing mouse WNT-medulloblastoma cells were orthotopically implanted into CD1-nude mice and monitored for tumour development via bioluminescent imaging. Upon tumour engraftment, mice were subjected to reduced SOC (radiotherapy and adjuvant vincristine) plus a clinically relevant dose of loratadine. Response and survival were compared to mice treated with full SOC (radiotherapy, vincristine, cisplatin, and etoposide). Mice treated with 2mg/kg/day of loratadine following reduced SOC demonstrated increased survival when compared to those treated with full SOC (p=0.02) along with a significant reduction in weight loss during treatment (p=<0.0001). This work suggests that loratadine, or other FIASMA compounds, may be good alternative adjuvant therapies for WNT-medulloblastoma. Using less toxic adjuvants could improve long-term outcomes through reducing therapeutic related toxicities for children with this devastating disease.

MEDB-33. THE LANDSCAPE OF ECDNA IN MEDULLOBLASTOMA

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Extrachromosomal circular DNA (ecDNA) is an important driver of aggressive cancers, including medulloblastoma (MB), the most common malignant pediatric brain tumor. To assess the clinical importance of ecDNA in MB, we applied computational methods to detect ecDNA in the genomes of a cohort of 468 MB patients and 31 MB model systems. Among patients, ecDNA was detected in 18% of tumors and carried a threefold greater risk of mortality. Affected genomic loci harbor up to hundredfold amplification of oncogenes including MYC, MYCN, TERT, and other novel putative oncogenes. Between sequential patient biopsies at initial diagnosis and subsequent relapse, we observed structural variation at ecDNA loci and generation of new ecDNA sequences. Among model systems, ecDNA was found in 19 of 31 genomes (61%). Although ecDNA was far more prevalent among MB models than patients, the ecDNA genomic sequences were conserved between most patient-derived xenograft (PDX) models and the human tumors from which they were made. To elucidate the functional regulatory landscapes of ecDNAs in MB, we generated transcriptional (RNA-seq), accessible chromatin (ATAC-seq), and chromatin interaction (Hi-C) profiles of 6 MB tumor samples. In each case, we identified regulatory interactions that cross fusion breakpoints on the ecDNA, representing potential "enhancer rewiring" events which may contribute to transcriptional activation of co-amplified oncogenes. To test this hypothesis, we are currently conducting in vitro CRISPRi screens targeting regulatory regions on the ecDNA of a MB cell line to determine whether these enhancers promote proliferation. Using single-cell sequencing, we have also begun exploring intratumoral heterogeneity of ecDNA in a p53-mutant SHH MB patient tumor and its corresponding PDX model. In summary, our study analyzes the frequency, diversity, and functional relevance of ecDNA across MB subgroups and provides strong justification for continued mechanistic studies of ecDNA in MB with the potential to uncover new therapeutic approaches.

MEDB-34. A VERY RARE CASE: MEDULLOBLASTOMA RELAPSE WITH BONE MARROW INFILTRATION IN A TODDLER

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We report about a female toddler congenitally deaf and diagnosed with a non-metastatic desmoplastic medulloblastoma (SHH activated, *TP53*-wt, variant in *LDB1* gene). No tumor predisposition syndrome was found. After complete tumor resection the patient was treated according to I-HIT-MED-Guidance protocol. Five months later an asymptomatic localised relapse (same histology, *PTEN* frameshift deletion, *TERT* mutation, *LDB1* mutation) detected by routine MRI was treated by complete resection, craniospinal irradiation and an antiangiogenic regimen adapted from the MEMMAT scheme including fenofibrate, thalidomide, celecoxib, topotecan, temozolomide, bevacizumab and intraventricular cytarabine. Before start of systemic treatment blood cell counts were normal. In the second cycle we had to interrupt chemotherapy due to a leukopenia while continuing the antiangiogenic treatment. In order to avoid relevant bone marrow toxicity chemotherapy doses were reduced. Nevertheless we had to stop the fourth cycle because of a severe pancytopenia. Same time the girl presented with fever, neck and leg pain. A full blood count showed: hemoglobin 6.92 g/dl, leukocytes 640/µl, platelets 8,000/µl. Suspecting an infection supported by the presence of a high CrP value of 230 mg/l the patient was treated with i.v. antibiotics. MRI showed an unspecific retropharyngeal soft tissue augmentation, a pleural effusion and high T2 signals in multiple vertebral bodies but no central tumor relapse. The bone marrow diagnostics revealed a diffuse medulloblastoma cell infiltration with the known *PTEN* frameshift deletion and *LDB1* mutation. The liquor was tumor-cell free. We report on an extremely rare case of an early local relapse of desmoplastic medulloblastoma progressing to a diffuse bone marrow infiltration in a toddler. The girl died due to therapy resistance 9 weeks after bone marrow relapse. It remains unclear whether the fatal course was related to the hereditary deafness syndrome and the molecular alterations of the tumor.

MEDB-35. RELATIONSHIP BETWEEN GENETIC PROFILE, HISTOLOGY, CLINICAL FEATURES AND LONG-TERM OUTCOME IN YOUNG CHILDREN MEDULLOBLASTOMA (YCMB) TREATED WITH UPFRONT HIGH DOSE CHEMOTHERAPY (HDCT) IN ITALY

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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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