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Nearly one-third of children with medulloblastoma, a malignant embryonal tumor of the cerebellum, succumb to their disease. Conventional response monitoring by imaging and cerebrospinal fluid (CSF) cytology remains challenging and a marker for measurable residual disease (MRD) is lacking. Here, we show the clinical utility of CSF-derived cell-free DNA (cfDNA) as a biomarker of MRD in serial samples collected from children with medulloblastoma (123 patients, 476 samples) enrolled on a prospective trial. Using low-coverage whole-genome sequencing, tumor-associated copy-number variations (CNVs) in CSF-derived cfDNA are investigated as an MRD surrogate. MRD is detected at baseline in 85% and 54% of patients with metastatic and localized disease, respectively. The number of MRD-positive patients decline with therapy, yet those with persistent MRD have significantly higher risk of progression. Importantly, MRD detection precedes radiographic progression in half who relapse. Our findings advocate for the prospective assessment of CSF-derived liquid biopsies in future trials for medulloblastoma.

MEDB-75. TREATMENT-INDUCED PULMONARY TOXICITY IN PATIENTS WITH MEDULLOBLASTOMA: A RETROSPECTIVE ANALYSIS ON 2 ITALIAN INSTITUTIONS' COHORTS

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BACKGROUND: Incidence of iatrogenic pulmonary toxicity is around 20%. Apart from bleomycin fibrosis, the role of lomustine, HD-thiotepa, autologous stem-cells transplantation (APBSCT) and their synergy with craniospinal irradiation (CSI) are unclear. To elucidate their role in lung-function impairment, we retrospectively evaluated 39 medulloblastoma patients treated at INT-Milan and OPBG-Rome. **METHODS:** 39 patients (17 females, median RT age 8 years) treated for localized (29) or metastatic (10) medulloblastoma in 2000-2020 and with spirometric assessment, were considered. Treatment included: SIOP-like-PNET IV (19), high-risk protocol (19), infant protocol without RT (1). CSI doses were: 23.4 Gy (20), 31.2 Gy (8), 36 Gy (6) and 39 Gy (4); 4 received protons and 34 photons (9 VMAT, 25 3D), 11 hyperfractionated-accelerated-RT; 33 had 6 median CCNU cycles; 6 APBSCT. **RESULTS:** Median follow-up: 98 months. All patients performed at least one spirometry at median 5 years after RT. Eight (20.6%) had mildly pathological spirometries, 8 Forced Vital Capacity (FVC%) <90%. RT age was not associated with FVC%/PEF% (p=0.319 and 0.405). A lower Peak Expiratory Flow (PEF%) was marginally associated to APBSCT group (p=0.062) with FVC% (≤90% vs >90%) similar but less significant (p=0.163). Median FVC%/PEF% were higher in the CCNU-group without reaching significance (p=0.436 and 0.062); this was a standard-risk group not receiving APBSCT nor higher RT doses. Even though the lung volume encompassed by 5-10 Gy isodoses was greater in VMAT vs 3D RT (p<0.001 and p=0.015), there were no significant differences in ventilatory parameters. FVC%/PEF% were negatively associated to CSI dose. Since no relevant lung volume is involved in high doses, a multifactorial etiology could be speculated. **CONCLUSIONS:** Preliminary data show no significant FVC%/PEF% reduction. Small sample size and differences in spirometry techniques impose larger cohorts accrual to elucidate potential treatment-induced pulmonary impairment in the pediatric population thus validating the use of spirometry during treatment/follow-up.

MEDB-76. EVALUATING THE B7-H3 CHECKPOINT IN MEDULLOBLASTOMA

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BACKGROUND: There is currently no curative therapy for recurrent/refractory MB. Novel approaches to MB include immunotherapy, such as targeting the immune checkpoint molecule B7-H3. B7-H3 is implicated in tumor metastasis and is highly expressed in MB. This study explores the ef-

fects of genetically knocking down B7-H3 in a murine model of recurrent/refractory medulloblastoma. **METHODS:** Murine MB cells were transduced with a CRISPR/Cas9 lentivirus to create a B7-H3 knockout. Knockout population was sorted twice via FACS by the AECOM flow cytometry CORE and confirmed by western blot and flow cytometry. Three healthy clones were used in subsequent studies, and compared to the wild type and the scramble control. IncuCyte live imaging technology was used to evaluate spheroid growth. Matrigel Boyden chambers were used to evaluate migration. Bulk RNA-seq was performed by the Yale University Core. **RESULTS:** B7-H3 knockout was successful in the murine MB model. Morphological differences were noted in the B7-H3 knockout cells. Spheroid formation assays show one of the clones with statistically slower growth kinetics compared to controls. Migration results are pending. RNA seq revealed similar clustering amongst knockouts, separate from controls with an enrichment in genes of morphologic development, WNT signaling and amoeboid migration. **CONCLUSIONS:** The morphologic changes in the B7-H3 knockouts suggest a potential growth differential. Although in vitro growth assays have shown mixed results regarding the effect of knocking out B7-H3 in spheroid formation, B7-H3 has been more directly implicated in migration and immune signaling. If migration is impaired, this will suggest that B7-H3 enhances malignant and metastatic potential in MB. Functional in vivo immune studies in syngeneic mice will investigate immune mediated effects of B7-H3 knockout in this tumor. If our studies support a role for B7-H3 in the development of MB, it may have important clinical implications, particularly for relapsed patients.

MEDB-77. METASTATIC MEDULLOBLASTOMA: RADIOLOGICAL FEATURES AND ITS CORRELATION WITH MOLECULAR SUBGROUPS AND DISSEMINATION PATTERN

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Medulloblastoma (MB) is the most frequent malignant childhood brain tumor. Four molecular subgroups have been described (WNT, SHH, group3, group4), which are associated with a different biological profile, prognosis, specific MRI characteristics and patterns of metastatic dissemination. We aimed to determine the imaging features of the metastatic MB and its molecular subgroup and their outcomes. Retrospective single-center analytic-observational study conducted from January 2004-January 2022 in a tertiary-care center. Pediatric patients with metastatic medulloblastoma at disease onset were included. We collected epidemiological and clinical characteristics, treatment received, and outcomes. The molecular subgroup was determined by its methylation profile. MRI were reviewed by the neuroradiologist. Sixty-three patients were diagnosed, 17 (26.9%) were metastatic. The median age at diagnosis was 5.1 years (range 2.1-17.5 years), 58.8% were male. According to histopathologic classification, fifteen patients (93.8%) were classic, 1 (6.3%) desmoplastic. Molecular subgroup analysis showed 2 WNT (12.5%), 1 SHH (6.3%), 3 (18.8%) group 3 (G3) and 5 (31.2%) group 4 (G4). Four patients (25%) were classified as G3/G4 and 1 (6.3%) as mixed. Five patients (29.4%) were M2 and 12 patients (70.6%) were M3 according to Chang staging system. The location in the cerebellar hemispheres was only observed in SHH patient while G3 tumors presented homogeneous contrast enhancement. All WNT, G3 and G4 were located in IV ventricle. We found no association between molecular subgroup and metastatic site (intracranial vs spinal, Fisher test, p=0.45). All patients presented with metastasis in the third ventricular infundibular recess were G4. Four patients died, all of them were G3 or G3/G4. Our results supported the literature previously reported. According to the MRI imaging features, the molecular medulloblastoma subgroups could be suggested. The presence of metastasis in the infundibular recess suggested MB group 4. However, the dissemination pattern could not be associated with any subgroup in our series.

MEDB-78. UNIFIED RHOMBIC LIP ORIGINS OF GROUP 3 AND GROUP 4 MEDULLOBLASTOMA

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Identification and characterization of lineage-specific beginnings of distinct medulloblastoma (MB) subgroups is a fundamental challenge in the field.