ously resilient against radiation therapy. Furthermore, wtIDH1 and IDH2 represent a unique target in radiation-resistant MB which has not previously been identified. Wild type IDH1/IDH2 are more recently shown to promote tumor proliferation and mediate metabolic reprogramming through the production of oncometabolites and substrates that functionally alter chromatin structure and gene transcription. We hypothesized that MYC modulation of wtIDH1/IDH2 facilitates metabolic reprogramming and promotes radiation-resistant cell populations. We show the change in the structural integrity of chromatin altered in radiation-resistant MB by metabolic adaptation and the effect of disrupting IDH1/IDH2 activity. We further compare these results to the chromatin profile of patient primary and matched relapsed MB samples at the single-cell level. We demonstrate that targeting IDH1/2 with chemical inhibitors suppresses MB cell growth. Our results disclose insights into the development of radiation resistance and provide a potential therapeutic target for the treatment of relapsed MYC-MB.

MEDB-71. MOLECULAR CHARACTERISATION OF GROUP 4 MEDULLOBLASTOMA IMPROVES RISK-STRATIFICATION AND ITS BIOLOGICAL UNDERSTANDING

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Group 4 (MB_{Grp4}) accounts for ~40% of medulloblastoma and the majority of non-WNT/non-SHH cases, yet its underpinning biology is poorly understood, and survival outcomes are not sufficiently explained by established clinicopathological risk factors. We investigated the clinical and molecular correlates of MB_{Grp4}, including second-generation methylation non-WNT/non-SHH subtypes (I-VIII) and whole chromosome aberration (WCA) subtypes (defined by chromosome 7 gain, 8 loss, and 11 loss; WCA-favourable risk [WCA-FR] ≥2 features, WCA-high risk [WCA-HR] ≤1 feature). A clinically-annotated MB_{Grp4} discovery cohort (n=378) was assembled from UK CCLG institutions, collaborating centres and SIOP-UKCCSG-PNET3/HIT-SIOP-PNET4 clinical trials. Contemporary molecular profiling integrating methylation/WCA subtypes and next-generation sequencing was performed. Survival modelling was carried out with patients >3 years old who received craniospinal irradiation (n=336). Association analysis confirmed relationships between methylation and WCA subtypes. Subtypes VI and VII were enriched for WCA-FR ($p<0.0001$) and aneuploidy, whereas subtype VIII was defined solely by i17q ($p<0.0001$). Whilst we observed an overall low mutational burden, WCA-HR harboured recurrent mutations in genes involved in chromatin remodelling ($p=0.007$). No gene-specific events were associated with disease risk, however integration of both methylation subtype and WCA groups enabled improved risk-stratification survival models that outperformed current schemes. The optimal MB_{Grp4}-specific model stratified patients into: favourable-risk (local disease, subtype VII or subtype VI with WCA-FR; 39/194 [20%], 97% 5-year PFS), very-high-risk (metastatic disease with WCA-HR; 71/194 [37%], 50% 5-year PFS) and high-risk (remaining patients; 84/194 [43%], 67% 5-year PFS). Findings were validated in independent cohorts. Comprehensive clinico-molecular assessment of MB_{Grp4} provides important understanding of its clinical and biological heterogeneity. Our novel MB_{Grp4} stratification scheme removes standard risk disease and identifies a favourable risk group (20% of MB_{Grp4}) with potential for therapy de-escalation. Current therapeutic strategies are insufficient for the very-high risk group (encompassing 37% of MB_{Grp4}), for whom novel therapies are urgently required.

MEDB-72. MOLECULAR CHARACTERIZATION OF MEDULLOBLASTOMAS IN A SINGLE INSTITUTION

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INTRODUCTION: The four molecular groups (WNT, SHH, Group 3 and Group 4) in medulloblastoma have been well established for the past decade. New subgroups within the four principal molecular groups have recently been discovered and recognized by WHO classification of Central Nervous System Tumours (5th edition). Subgroups were reported to have distinct somatic copy-number aberrations and clinical outcomes. This further classification could be helpful to refine prognostication and potentially provide risk stratification for treatment planning. AIM: To interrogate archival medulloblastoma samples using Oncoscan Microarray Assay, correlate with clinical features and consider the assay for clinical use. METHODS: Thirty-one archival samples with histological diagnosis of medulloblastoma and molecular grouping results from NanoString were retrieved and evaluated with Oncoscan Microarray Assay. Twenty-six were subjected to DNA methylation profiling to compare the results. Eight cases also had molecular data from next-generation sequencing (NGS) done with the in-house Ampliseq Childhood Cancer Panel. Correlation was made with clinical characteristics and outcomes of these 31 patients. RESULTS: OncoScan microarray showed distinct differences in the copy number profiles of the 31 medulloblastoma samples. Seventeen samples could be further classified into one of 12 subgroups. However, further subgrouping was challenging without first determining the main molecular group especially amongst non-WNT/SHH tumours. DNA methylation results provided corroboration with the Oncoscan subgrouping results in 25 of 26 samples. NGS panel detected additional genetic alterations in 5 of 8 samples. CONCLUSIONS: Oncoscan Microarray Assay showed potential in providing additional molecular information for further subgrouping of medulloblastoma, but was insufficient for determining the main molecular groups. Moving forward, molecular characterization could instead be done through use of NGS panel and DNA methylation, which provides tumour epigenetic profiling on top of copy number variants. These could be used alongside the NanoString platform, which is performed routinely for all medulloblastomas at our centre.

MEDB-73. LIPID METABOLISM AS A THERAPEUTIC VULNERABILITY IN BET INHIBITOR-RESISTANT MEDULLOBLASTOMA

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MYC-driven medulloblastomas are a particularly devastating group of pediatric brain tumors that exhibit resistance and continued progression despite standard of care treatments. Our preclinical work identified BET-bromodomain inhibitors as a potentially promising new class of drugs for children with medulloblastoma and other MYC-driven cancers, providing rationale to evaluate these agents in clinical trials. However, treatment with BET inhibitor (BETi) alone is unlikely to be sufficient to cure, with most tumors evolving to acquire resistance to single-agent targeted therapies. We applied an integrative genomics approach to identify genes and pathways mediating BETi response in medulloblastoma. These studies revealed that MYC-driven medulloblastoma cells with acquired resistance to BETi reinstate transcription of essential genes suppressed by drug and exhibit changes in cell state and new vulnerabilities not present in drug-sensitive cells. We now have a growing body of evidence showing that BET inhibition downregulates the expression of key lipid metabolism genes and metabolism-related signaling pathways, and that medulloblastoma cells with adaptive resistance to drug differentially express and exhibit preferential dependency on specific lipid metabolic genes and transcriptional regulators. Our studies explore the possibility of exploiting these metabolic vulnerabilities to overcome BETi resistance and provide a more efficacious upfront therapy.

MEDB-74. SERIAL ASSESSMENT OF MEASURABLE RESIDUAL DISEASE IN MEDULLOBLASTOMA LIQUID BIOPSIES

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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: 23.4Gy(20), 31.2Gy(8), 36Gy(6) and 39Gy(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 VMATvs3D 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 effects 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. METASTASIC 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.