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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.

Genetically engineered mouse models and cross-species transcriptomics have provided mounting evidence of discrete, subgroup-specific developmental origins. Likewise, murine single-cell transcriptional atlases of cerebellar development have recently provided further clues into MB subgroup origins, particularly for poorly defined Group 3 and Group 4-MB. However, initial studies were underpowered to characterize rare populations and lacked robust validation, resulting in incomplete findings. Herein, we leveraged a large harmonized murine cerebellar atlas, targeted lineage enrichment, and integrative multi-omic strategies to deeply dissect MB origins. Isolation of spatially and temporally discrete developmental trajectories of key glutamatergic lineages born out of the murine upper rhombic lip provided an enhanced reference for mapping MB subgroup origins, especially for Group 3 and Group 4-MB. However, human-specific anatomic and cellular complexity, particularly within the rhombic lip germinal zone complicated murine-derived inferences. Further tumor-normal integrations using a novel single-cell atlas of the human fetal cerebellum, companioned with laser-capture micro-dissected transcriptional and epigenetic datasets, reinforced developmental insights extracted from candidate murine cerebellar lineages. Characterization of compartment-specific transcriptional signatures identified in the human upper rhombic lip implicated convergent cellular correlates of Group 3 and Group 4-MB, suggestive of a common developmental trajectory underlying their ancestry. Systematic imaging review and 3D summarization of a large clinical trial series of patient tumors, coupled with our advanced insights into developmental signatures, substantiated subgroup-specific tumor location patterns observed at diagnosis. Together, our results strongly implicate a common lineage trajectory of the upper rhombic lip as the probable origin of Group 3 and Group 4-MB. These important findings provide unprecedented opportunities to explore context-dependent mechanisms of MB pathogenesis and will foster generation of improved preclinical models that more faithfully recapitulate tumor biology.

MEDB-79. MYC-DRIVEN UPREGULATION OF THE *DE NOVO* SERINE AND GLYCINE PATHWAY IS A NOVEL THERAPEUTIC TARGET FOR GROUP 3 MYC-AMPLIFIED MEDULLOBLASTOMA

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Despite advances in the molecular sub-classification and risk-stratification of medulloblastoma (MB), a subset of tumours remain refractory to current multimodal therapies. Group 3 (MB_{Group3}) patients represent around 25% of MBs, and amplification and elevated expression of *MYC* in this group correlates with dismal clinical outcomes. Since direct targeting of MYC remains elusive, understanding and exploiting metabolic dependencies in MYC-amplified MB_{Group3} may reveal novel therapeutic opportunities. We engineered three independent regulable *MYC*-amplified MB_{Group3} cell-based models, each harbouring doxycycline-inducible anti-MYC shRNAs (two independent species) or a non-silencing shRNA control. In all three models, MYC knockdown (KD) revealed persistent MYC-dependent cancer phenotypes, reduction in proliferation and cell cycle progression. We utilised ^{1}H high-resolution magic angle spectroscopy (HRMAS) and stable isotope-resolved metabolomics to assess changes in intracellular metabolites and pathway dynamics when *MYC* expression was modulated. Profiling revealed consistent MYC-dependent changes in metabolite concentrations across models. Notably, glycine was consistently accumulated following *MYC* KD suggesting altered pathway dynamics. ^{13}C-glucose tracing further revealed a reduction in glucose-derived serine and glycine (*de novo* synthesis) following MYC KD which was attributable to lower expression of PHGDH, the rate-limiting enzyme of this pathway. Furthermore, in human primary tumours, elevated expression of PHGDH was associated with *MYC* amplification and poorer survival outcomes. MYC expressing cells showed greater sensitivity to pharmacological inhibition of PHGDH compared to MYC KD (MB_{Group3}) and MB_{SHH} subgroup cell lines *in vitro*. Critically, targeting PHGDH *in vivo*, using MYC-dependent xenografts and genetically engineered mouse models, consistently slowed tumour progression and increased survival. In summary, metabolic profiling has uncovered MYC-dependent metabolic alterations and revealed the *de novo* serine/glycine synthesis pathway as a novel and clinically relevant therapeutic target in *MYC*-amplified MB_{Group3}. Together, these findings reveal metabolic vulnerabilities of *MYC*-amplified MB_{Group3} which represent novel therapeutic opportunities for this poor-prognosis disease group.

MEDB-80. CDK8 PROMOTES STEMNESS OF MYC-DRIVEN MEDULLOBLASTOMA

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Cyclin-dependent kinase 8 (CDK8) belongs to the transcription-related cyclin dependent protein kinase family. CDK8 and cyclin C associate with the mediator complex to regulate gene transcription. Although CDK8 has been shown to be implicated in the malignancy of several types of cancer, its functional role and mechanism in medulloblastoma remains largely unknown. Here, we demonstrate how CDK8 plays an essential role in maintaining stemness and tumorigenicity in medulloblastoma stem cell. CDK8 inhibition suppresses stem cell associated signaling in medulloblastoma cells and inhibits tumor cell self-renewal. Additionally, CDK8 is amplified in MYC-driven medulloblastoma, is positively correlated with c-MYC expression in human medulloblastoma specimens and associates with poor survival in patients. Using cut&run assay, we found CDK8 associates with MED1 to activate transcription of MYC target genes. CDK8 attributes to MYC-driven transcriptional programs mediating DNA repair. Pharmaceutic inhibitors and genetic depletion result in cessation of tumor growth in xenograft mouse models and increase in apoptosis and DNA damage. Collectively, our studies establish the selective inhibition of CDK8 inhibition as a viable therapeutic strategy in MYC-driven medulloblastoma.

MEDB-81. COMBINED INHIBITION OF CDK11 AND EZH2 RESULTS IN REGRESSION OF MYC-AMPLIFIED MEDULLOBLASTOMA

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We explored an shRNA library screen on 20 cyclin-dependent kinases to establish cyclin-dependent kinase 11 (CDK11) as a critical mediator in MYC-driven medulloblastoma. The effect and molecular mechanism of CDK11 in the proliferation and growth of medulloblastoma were investigated in vitro. Pharmaceutic inhibitors and genetic depletion of CDK11 resulted in cessation of tumor growth in xenograft mouse models. Through combination chemical screening, we identified that 5-FU enhanced the apoptosis which induced by inhibition of CDK11 in medulloblastoma cells. In addition, we found CDK11 is a significant candidate kinase participating in the negative control of Wnt/b-catenin signaling. Down-regulation of CDK11 led to the accumulation of Wnt/β-catenin signaling receptor complexes through activation of transmembrane Frizzled (FZD) receptors which is suppressed by H3K27Me3. RNASeq and cut&run revealed that Cdk11 and mediator associated Cdk8 kinase regulate a common set of genes. Lack of Cdk8 and Cdk11 impaired Ezh2 recruitment and the establishment of histone H3 lysine 27 tri-methylation. We concluded that combined EZH2 and CDK8/CDK11 inhibitors treatment concurrently activated Wnt signaling may be an effective treatment for Group 3 medulloblastoma.

MEDB-82. EXPLORING CELL-CELL COMMUNICATION NETWORKS IN MEDULLOBLASTOMA USING SINGLE-CELL GENOMICS

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Medulloblastoma is a high-risk embryonal brain tumor arising in the cerebellum. Genomic profiling has revealed a striking molecular heterogeneity between medulloblastoma patients, yet treatment regimens are mostly uniform. Many children with medulloblastoma die from their disease and surviving patients often face severe long-term side effects, highlighting an urgent need for more effective treatment options. We and others have recently identified pronounced intra-tumoral heterogeneity and defined cellular hierarchies within medulloblastoma tumors. The functional role of these cellular hierarchies remains unknown. We now hypothesize the existence of an inter-cellular communication network that is maintained by receptor/ligand interactions. To test our hypothesis, we use our medulloblastoma single-cell RNA sequencing dataset of 25 patients, as well as bulk RNA sequencing, DNA methylation array, and genome sequencing data across molecular subtypes. Single-cell RNA sequencing data are analyzed to dissect cell compartments characterized by high expression of potentially oncogenic receptors and their respective ligands. Consequently, cell type-specific roles in auto- or paracrine signal transduction within the cellular community are explored. We further investigate downstream oncogenic signaling pathways by approximating transcription factor activity and explore genetic and epigenetic