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BACKGROUND: Diffuse intrinsic pontine gliomas (DIPGs) are lethal pediatric brain tumors with no curative therapies. Inhibitor of DNA binding (ID) proteins are key regulators of gene differentiation during embryogenesis. Previous work has shown that *H3F3A* and *ACVR1* mutations increase ID1 expression in cultured astrocytes, but this has not been validated in human DIPG, nor has the regulation and targetability of ID1 been explored in DIPG. RESULTS: Analysis of post-mortem tissue and multiple human datasets showed ID1 to be elevated in DIPG, and to correlate with reduced survival. In a multi-focal autopsy of a DIPG case, we also found ID1 expression to be heterogeneous and to correlate with tumor invasion. Chromatin immunoprecipitation qPCR (ChIP-qPCR) revealed elevated H3K27ac and low H3K27me3 at ID1 regulatory regions (enhancers/promoters) in DIPG tissue compared to normal brain, regardless of H3 or *ACVR1* mutation status. Analysis of publicly-available ISH and ChIP-sequencing data of developing murine brains revealed H3K27ac at ID1 enhancers to be elevated in the prenatal hindbrain compared to prenatal forebrain and midbrain, and all postnatal brain regions. ID1 shRNA-mediated knockdown of primary human H3K27M DIPG cells (DIPG007) significantly reduced invasion and migration. We also treated DIPG007 cells with cannabidiol (CBD) and found reduced viability at clinically relevant dosing (IC50=2.4 uM) with dose-dependent reduction in ID1 protein. CONCLUSIONS: These findings indicate that a multifactorial (genetic and regionally-based) epigenetic upregulation of *ID1* drives DIPG invasiveness and is targetable with CBD. ID1 knockdown and CBD treatment experiments in murine models of DIPG are ongoing.

DIPG-60. PILOT STUDY OF CIRCULATING TUMOR CELLS IN PEDIATRIC HIGH GRADE BRAIN TUMORS

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BACKGROUND: Despite its increasing use, circulating tumor cells (CTCs) have not been studied in pediatric brain tumors. METHODS: Cell surface vimentin (CSV) is a marker for CTC detection. We developed an automated CSV-based CTC capture method for pediatric brain tumor using the Abnova Cytoquest platform. PBMCs isolated from blood samples from 52 brain tumor patients were processed to isolate CSV^+ CTCs. Captured cells were then stained for CSV and CD45 and scanned to determine the number of CTCs. DIPG samples were additionally examined for H3K27M expression on CSV^+ cells. Long term cancer survivors were used as a control cohort. RESULTS: 86.4% of all the samples exhibited between 1–13 CSV+ CTCs, with a median of 2 CSV+ CTCs per sample. Using a value of ≥ 1 CTC as a positive result, the sensitivity and specificity of this test was 83.05% and 60.0% respectively. 19 DIPG samples were analyzed and 70% (13 samples) were positive for 1–5 CTCs. Five of these 7 positive CSV+ CTCs DIPG samples were also positive for H3K27M mutations by immunohistochemistry (71%). Mean survival in days for the CTC positive and negative DIPG samples were 114 and 211 days, respectively (p= 0.13). CONCLUSION: This is the first study of CTCs in pediatric CNS tumors using an automated approach. Patients with brain tumors can exhibit CSV+ CTCs within peripheral blood. The use of specific molecular markers such as H3K27M can improve the diagnostic capability of liquid biopsies and may enable future disease assessment for personalized therapy.

DIPG-61. RESCUE REGIMENS AFTER BIOMEDE: POSSIBLE INFLUENCE ON OS ASSESSMENT

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BIOMEDE is a multicentric randomized phase II trial to evaluate in DIPG the OS of patients treated with dasatinib, erlotinib or everolimus. The OS is the result of the first line treatment but it could also be affected by re-irradiation and the second line treatment received after progression, especially in case of a possible crossover outside of the trial. This preliminary analysis focuses on the first patients enrolled at Gustave Roussy (n=37). The median age at diagnosis was 7 years, median interval from diagnosis to progression and median survival after progression were 7 (1–20) and 2 (0–13) months respectively. Initial treatment was everolimus for 13, dasatinib for 20, erlotinib for 4 patients. The most frequent targetable molecular alterations were mTOR pathway in 6, PDGRFA in 4, ACVR1 in 3 patients. Out of the 31 patients who relapsed and were evaluable, 18 and 13 had a median survival < 3 and > 3 months respectively. At relapse patients have received different types of therapies, in 6 cases matching the molecular profile of the tumour obtained by sequencing. At progression, seven patients switched from dasatinib to mTOR inhibitors and 2 patients switched from everolimus to dasatinib. Patients with OS after progression > 3 months had higher rate of reirradiation (77% vs 5%), steroid weaning (69% vs 33%) and Lansky/Karnowsky > 50% (85 vs 67%). Extended results on the entire cohort will be presented. It will be important to consider the distribution of reirradiation to interpret the results of the randomisation on OS.

DIPG-62. PRECLINICAL EVALUATION OF IMIPRIDONE-BASED COMBINATION THERAPIES IN PEDIATRIC H3K27M MUTANT DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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Imipridones induce apoptosis in cancer via p53 independent upregulation of TNF-related apoptosis inducing ligand (TRAIL) pathway and its proapoptotic receptor DR5. ONC201, a first-in-class imipridone, is being evaluated alone and with radiotherapy for children with H3K27M mutant diffuse glioma. We sought to determine if ONC201 and its imipridone analogs (ONC206, ONC212) are synergistic with other chemotherapy agents. Seven patient-derived DIPG cell lines, six H3.3K27M mutant (SU-DIPG-IV, SU-DIPG-13, SU-DIPG-25, SU-DIPG-27, SU-DIPG-29, SF8628) and one H3.1K27M mutant (SU-DIPG-36) were grown in culture and exposed to ONC201, ONC206, and ONC212 alone and in combination with histone de-acetylase inhibitors (HDACi) or etoposide. A dose-dependent response to ONC201, ONC206, and ONC212 was demonstrated in all cell lines, with mean IC50 values of 1.46 μM, 0.11 μM, and 0.03 μM respectively. ONC206 and ONC212 induced apoptosis measured by increased expression of cleaved PARP and ISR by increased expression ATF4. In two cell lines, synergy studies revealed combination indices (CI) < 1 for ONC206 and etoposide, with a best CI of 0.62 in SU-DIPG-IV and 0.46 in SU-DIPG-25. Synergy was also observed between ONC201 and etoposide (CI 0.46) and ONC201 and panobinostat (CI 0.01). Imipridones and analogs were superior to panobinostat and etoposide in triggering apoptosis as measured by sub-G1 phase content. Additional synergy and mechanistic analyses are ongoing and will be reported. Our results suggest that H3K27M mutant DIPG cells demonstrate increased sensitivity to imipridone analogs (ONC206 and ONC212) when compared to ONC201. Combinational strategies with etoposide or HDACi should be considered for clinical translation.

DIPG-63. LOSS OF THE H4 LYSINE METHYLTRANSFERASE KMT5B DRIVES INVASION / MIGRATION BY DEPLETING H3K27ME3 AT LOCI OTHERWISE RETAINED IN H3K27M MUTANT DIPG CELLS

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Diffuse intrinsic pontine glioma (DIPG) and other diffuse midline glioma (DMG) are characterised by K27M mutations in histone H3 variants. The major functional consequence is a global loss of the repressive mark H3K27me3, causing a raft of transcriptional changes promoting tumorigenesis, although certain key loci retain trimethylation, such as *CDKN2A/B*. We recently identified subclonal loss-of-function mutations in the H4 lysine methyltransferase *KMT5B* to be associated with an enhanced invasion/migration, but the mechanism by which this occurred was unclear. Here we show by ChIP-seq using patient-derived subclonal DIPG models and CRISPR-Cas9 depletion that loss of KMT5B (or KMT5C) causes a paradoxical increase in global levels of H4K20me3 in promoters and regulatory regions, only ablated by knocking out both enzymes. Loss of KMT5B alone further causes loss of the majority of otherwise retained H3K27me3 loci in DIPG cells, although *CDKN2A/B* itself was spared. De-repression occurred at bivalent loci marked by H3K4me3 and had elevated gene expression by RNAseq; these were significantly enriched for genes involved in chromatin remodelling and invasion/migration, the latter including *MMP9/MMP24*.