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There is no standard of care for cerebrospinal (CSF) diversion in children with diffuse intrinsic pontine glioma (DIPG), nor understanding of survival impact. We evaluated CSF diversion characteristics in children with DIPG to determine incidence, indications and potential impact on survival. Data was extracted from subjects registered in the International DIPG registry (IDIPGR). IDIPGR team personnel obtained clinical and radiographic data from the registry database and when appropriate, abstracted additional data from individual medical records. Univariable analyses were performed using the Fisher's exact test or Wilcoxon rank sum test. Survival was estimated using the Kaplan-Meier method. Evaluable patients (n=457) met criteria for DIPG diagnosis by central radiology review. Ninety-two patients (20%) had permanent CSF diversion. Indications for permanent diversion were hydrocephalus (41%), hydrocephalus and clinical symptoms (35%), and clinical symptoms alone (3%). Those with permanent diversion were significantly younger at diagnosis than those without diversion (median 5.3 years vs 6.9 years, p=0.0002), otherwise no significant differences in gender, race, or treatment were found. The progression-free and overall survival of those with permanent CSF diversion compared to those without permanent diversion was 4.5 and 10.9 months vs 6.9 and 11.2 months, respectively (p=0.001, p= 0.4). There was no significant difference in overall survival in patients with or without permanent CSF diversion among a large cohort of DIPG patients. Patients without permanent diversion had significantly prolonged progression free survival compared to those with permanent diversion. The qualitative risks and benefits of permanent CSF diversion need to be further evaluated.

DIPG-56. EXPLORATION OF TUMOR/STROMA INTERACTIONS IN DIPG XENOGRAFT BY SPECIES-SPECIFIC RNA-SEQ DECONVOLUTION INDICATES A ROLE OF MICROGLIA CELL IN DIPG DEVELOPMENT

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Diffuse Intrinsic Pontine Glioma (DIPG) and more largely Diffuse Midline Gliomas H3 K27M-mutant (DMG) harbor a unique property of infiltration. Our objective is to elucidate/describe the cellular and molecular determinants of micro-environmental modifications resulting from the tumour/stroma dialogue as it might provide pro-invasive conditions that favour the development of the disease. To this end, we performed RNA-seq analyses to characterize exhaustively the bidirectional molecular modifications of the stroma/tumour in DIPG xenograft models. Gene expression changes in murine microenvironment compartment were investigated as continuous or semi-continuous traits of tumor load by measuring transcriptome in zone with high *vs*. low infiltration. We observed substantial modulations in gene expression in the microenvironment associated with increasing tumor cell content, pointing to a modification of the macrophage/microglial infiltrate. The expression or overexpression of several modulated genes was validated by IHC in the stroma of DMG primary tumors. Among them, overexpression of the cytokine CCL3 was confirmed, reflecting the activation status of microglial cells. Moreover, we observed in patients that the density of IBA-1 positive microglial cells increases according to the extent of tumor infiltration and that a significant part of them harbor a mitotic status, supporting their interaction with DMG cells. The involvement of this interaction in DMG development needs further evaluation and might represent opportunity to slow down DIPG extension.

DIPG-57. TRANSCRIPTOMIC AND PROTEOMIC ANALYSES OF DIPG RESPONSE TO ONC201

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Diffuse Intrinsic Pontine Glioma (DIPG) is an incurable pediatric brain tumor. Current standard of care has shown no improvements in survival. Here, we report our study of ONC201, a first-in-class small molecule developed by Oncoceutics, Inc., against a panel of DIPG cells *in vitro* and in mouse orthotopic models. ONC201 inhibits signaling through dopamine receptor D2 (DRD2), a G protein-coupled receptor (GPCR). MTT assays revealed a delayed but more robust response to ONC201, as measured by IC50 values, in DIPGs with histone H3.3-*K27M* expression compared to cells expressing wildtype (WT) or *K27M* mutant histone H3.1. Interestingly, transcriptomic profiling identified an association of this response delay with an elevation of genes controlling the cellular unfolded protein response, lysosomal and vacuole organization, and a decline in nucleic acid biosynthetic genes. These cells were also more committed to neuronal and oligodendrocytic lineage specification. By contrast, WT-H3 DIPGs that survived ONC201 treatment were stem-like and exhibited altered expression of genes controlling cell proliferation and apoptosis induction, respectively. Single cell proteomics validated the increase in anti-apoptotic proteins in these cells. Intraperitoneal administration of ONC201 for 7-weeks in mice bearing pontine xenografts of histone H3.1-*K27M* mutant DIPGs, caused a complete blockade of tumor growth relative to untreated controls. However, identical treatment of animals with forebrain tumors resulted only in a partial reduction in tumor burden, suggesting that the tumor microenvironment may be involved in the differential effect. These data indicate that tumor intrinsic and extrinsic factors may contribute to the response of DIPG tumors to ONC201.

DIPG-58. HISTONE H3 WILD-TYPE DIPG/DMG OVEREXPRESSING EZHIP EXTEND THE SPECTRUM OF DIFFUSE MIDLINE GLIOMAS WITH PRC2 INHIBITION BEYOND H3-K27M MUTATION

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Diffuse midline gliomas (DMG) H3 K27M-mutant were introduced in the 2016 WHO Classification unifying diffuse intrinsic pontine gliomas (DIPG) and gliomas from the thalamus and spinal cord harboring a histone H3-K27M mutation leading to Polycomb Repressor Complex 2 (PRC2) inhibition. However, few cases of DMG tumors presenting a H3K27 trimethylation loss, but lacking an H3-K27M mutation were reported. To address this question, we combined a retrospective cohort of 10 patients biopsied for a DIPG at the Necker Hospital or included in the BIOMEDE trial (NCT02233049) and extended our analysis to H3-wildtype (WT) diffuse gliomas from other midline locations presenting either H3K27 trimethylation loss or *ACVR1* mutation from Necker, ICR, the HERBY trial, the INFORM registry study and the St. Jude PCGP representing 9 additional cases. Genomic profiling identified alterations frequently found in DMG, but none could explain the observed loss of H3K27 trimethylation. Similar observations were previously made in the PF-A subgroup of ependymoma, where the H3K27me3 loss resulted from *EZHIP/CXorf67* overexpression rather than H3-K27M mutations. We thus analyzed *EZHIP* expression and observed its overexpression in all but one H3-WT DMGs compared to H3-K27M mutated tumors (EZHIP negative). Strikingly, based on their DNA methylation profiles, all H3-WT DMG samples analyzed clustered close to H3-K27M DIPG, rather than *EZHIP* overexpressing PF-A ependymomas. To conclude, we described a new subgroup of DMG lacking H3-K27M mutation, defined by H3K27 trimethylation loss and EZHIP overexpression that can be detected by IHC. We propose that these EZHIP/H3-WT DMGs extend the spectrum of DMG with PRC2 inhibition beyond H3-K27M mutation.

DIPG-59. UPREGULATION OF PRENATAL PONTINE ID1 SIGNALING IN DIPG

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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*.