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 (35%). Those with permanent diversion were significantly younger at diagnosis than those without diversion (median 5.3 years vs 6.7 years, p=0.01). There was no survival benefit in patients with or without permanent CSF diversion across 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 MICRÓGLIA CELL IN DIPG DEVELOPMENT**

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Diffuse Intrinsic Pontine Glioma (DIPG) and more largely Diffuse Midline Gliomas (DMG) harbor a unique property of infiltrative tumor growth. 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 zones with high or 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 mutated status supporting their interaction with DMG cells. The involvement of this interaction in DMG development needs further evaluation and might represent a potential therapeutic target.

**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 Oncocure, Inc., against a panel of DIPG cells in vitro and in immunodeficient mouse models. ONC201 inhibits signaling through dopamine receptor D2 (DR2), a G protein-coupled receptor (GPCR). MT assay 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 oligodendroglial lineages. Alternatively, impact on cell cycle progression in DIPG cell lines was not observed. In conclusion, 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 cell lines. Intraperitoneal administration of ONC201 in 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, single treatment of animals with forebrain tumors resulted only in a partial reduction in tumor burden, suggesting that tumor microenvironment may be involved in the differential effects. 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 H3PCC2 INHIBITION TO H3-WT, K27M DIPGs**

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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 H3K27M 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) DIPGs otherwise no significant differences in gender, race, symptoms alone (3%). Those with permanent CSF diversion were significantly older (median 5.3 years vs 6.3 years, p=0.4). There was no significant difference in overall survival by 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 (35%). Those with permanent diversion were significantly younger at diagnosis than those without diversion (median 5.3 years vs 6.7 years, p=0.01). There was no survival benefit in patients with or without permanent CSF diversion across 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-59. UPREGULATION OF PRENATAL PONTINE D1 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-tauopathy 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 BH and ChIP-sequencing data of developing mouse 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 camptothecin (CBD) and found reduced viability at clinically relevant dosing (IC50=2.4 μM) 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 mouse 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 for 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 per sample. When analyzed for CSV+ cells with camptothecin (CBD) and found reduced viability at clinically relevant dosing (IC50=2.4 μM) with dose-dependent reduction in ID1 protein. 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 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 COMBINATION THERAPIES IN PEDIATRIC H3K27M MUTANT DIFFUSE INTRINSIC PONTINE GLIOMA (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-tauopathy 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 BH and ChIP-sequencing data of developing mouse 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 camptothecin (CBD) and found reduced viability at clinically relevant dosing (IC50=2.4 μM) 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 mouse models of DIPG are ongoing.

DIPG-62. PRECLINICAL EVALUATION OF IMPRINDONE-BASED COMBINATION THERAPIES IN PEDIATRIC H3K27M MUTANT DIFFUSE INTRINSIC PONTINE GLIOMA (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-tauopathy 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 BH and ChIP-sequencing data of developing mouse 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 camptothecin (CBD) and found reduced viability at clinically relevant dosing (IC50=2.4 μM) 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 mouse models of DIPG are ongoing.

DIPG-63. LOSS OF THE H4 LYSINE METHYLTRANSFERASE KMT5B DRIVES INVASION / MIGRATION BY DELETING H3K27ME3 AT LOCI OTHERWISE RETAINED IN H3K27M MUTANT DIPG CELLS
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BACKGROUND: Diffuse intrinsic pontine gliomas (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 tumorgenesis, 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. The further 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/MMP2.