DIPG-52. PHASE I CLINICAL TRIAL OF ONC201 IN PEDIATRIC H3 K27M-MUTANT GLIOMA OR NEWLY DIAGNOSED DIPG
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H3 K27M-mutant gliomas often manifest as midline gliomas, have a dismal prognosis, and have no effective treatments. ONC201 efficacy has been shown in high-grade glioma preclinical models and durable responses with single agent ONC201 have been reported in adults with recurrent H3 K27M-mutant gliomas. These observations led to a Phase I pediatric clinical trial of ONC201 dosed by body weight. This multi-center, open-label, 3 + 3 dose-escalation and dose-expansion clinical trial (NCT03416330) for H3 K27M-mutant glioma or non-biopsied DIPG has 6 arms; arms A and E determine the RP2D in pediatric post-radiation (recurrent or not-recurrent) H3 K27M-mutant glioma patients with ONC201 administered as an oral capsule as well as a liquid formulation, respectively. Both arms have completed accrual. The study is currently enrolling new patients, ONC201 as a single agent in patients with progressive H3K27M mutant tumors following irradiation (excluding DIPG/spinal cord tumors) are currently enrolling patients. ONC201 as a single agent or in combination with radiation (arm B) is clinically significant therapeutic effect in H3F3A and PDGFA DIPG overexpression attenuates g-H2AX formation and suppresses apoptosis and cell-cycle arrest in response to radiation treatment. Deep scale phosphoproteomics analyses reveal DNA-damage and cell cycle pathways to be most significantly associated with PPM1D. Furthermore, preliminary analysis of genome-wide loss-of-function CRISPR/Cas9 screens in isogenic GFP and PPM1D overexpressing mouse neural stem cells reveal differential dependency on DNA-damage response genes in the PPM1D overexpressing cells. Consequently, ONC201 is a promising agent in progressive H3F3A and PDGFA DIPG, and will be further evaluated in a randomized phase II trial.

INTRODUCTION: We have previously found that up to 15% of all DIPGs harbor mutations in PPM1D, resulting in the expression of an activated and truncated PPM1D (PPM1Dtr). Here we evaluate the mechanisms through which PPM1Dtr enhances glioma formation in vitro and in vivo models of PPM1D-mutant DIPGs and applied quantitative proteomic and functional genomic approaches to identify pathways altered by PPM1Dtr. The development of PPM1Dtr as a clinical therapeutic is limited by toxicity. We find ectopic expression of PPM1Dtr to be sufficient to enhance glioma formation and to be necessary in PPM1D-mutant DIPG cells. In addition, endogenous truncation of PPM1D is sufficient to enhance glioma formation in the presence of mutation H3F3A and PDGFA. PPM1Dtr overexpression in DIPG cells is associated with increased cell death and in vivo models of PPM1D-mutant DIPG cells. In addition, endogenous truncation of PPM1D is sufficient to enhance glioma formation in the presence of mutation H3F3A and PDGFA. PPM1Dtr overexpression in DIPG cells is associated with increased cell death and in vivo models of PPM1D-mutant DIPG cells.
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 (IDPGR). IDPGR 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 6.8 years, p=.004). 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
DECONVAULTION 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 infiltrating the pons. 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 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. Moreover, we observed in patients that the overexpression of the cytokine CCL3 was confirmed, reflecting the activation status of microglial cells. Moreover, we observed in patients that the overexpression of the cytokine CCL3 was confirmed, reflecting the activation status of microglial cells. Moreover, we observed in patients that the overexpression of the cytokine CCL3 was confirmed, reflecting the activation status of microglial cells. Moreover, we observed in patients that the overexpression of the cytokine CCL3 was confirmed, reflecting the activation status of microglial cells.

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 on ONC201, a first-in-class small molecule developed by Oncoceutics, Inc., against a panel of DIPG cells in vitro and in xenograft models. ONC201 inhibits signaling through dopamine receptor D2 (DR2), a G protein-coupled receptor (GPCR). MTX 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 oligodendroglial lineage, impacting on cell survival. In contrast, WT-H3 DIPGs that survived 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. Intraptieternal 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 OVEREXPRESSION OF EZHIP EXTEND THE SPECTRUM OF DIFFUSE MIDLINE GLIOMAS WITH H3PCC2 INHIBITION ELICITING A COMPROMISED RESPONSE IN H3K27M MUTANT DIPG
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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 Polycymb 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 (NC02233049) and extended our analysis to H3-wildtype (WT) DIPGs. We thus investigated other midline locations presenting either H3K27 trimethylation loss or ACRV1 mutation from Necker, ICR, the HERBY trial, the INFORM registry study and the St. Jude PCGF presenting 9 additional cases. Genomic profiling identified alterations frequently found in DMG, but not consistently 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 DIPGs, 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.