(DIPGs), a pediatric glioma with 2-year survival rate of less than 10%. *ACVR1*mutations frequently coincide with activating *PIK3CA* or *PIK3R1* mutations, indicating a potential cooperative effect of BMP and PI3K signaling in gliomagenesis. We used genetically engineered mice with inducible knock-in of *Acvr1*^{R206H} or *Pik3ca*^{E545K} alleles, such that cre-mediated recombination resulted in expression of the gain of function mutated genes from their endogenous promoters at physiological levels. Cre-mediated deletion in *GFAP-CreER;Pik3caE545K/+;p53cKO* mice (*Pik3ca;p53*) mediated *Trp53* deletion and expression of *Pik3caE545K* in glial progenitors, and spontaneously induced high-grade glioma (HGG) in mice with complete penetrance. Heterozygous knock-in of the *Acvr1R206H* allele accelerated tumorigenesis and impaired survival in *Pik3ca;p53* mice (*Acvr1;Pik3ca;p53*). Transcriptomic analysis of *Acvr1;Pik3ca;p53* tumors compared to *Pik3ca;p53* littermate controls, as in patient-derived tumors, revealed broad molecular signatures associated with cell fate commitment and chromosome maintenance. Pharmacologic inhibition of ACVR1 was sufficient to impair growth in human patient-derived DIPG cell lines. Together, our studies show that ACVR1 activation promotes tumor growth in spontaneous mouse HGG and patient-derived DIPG cells, suggesting that ACVR1 inhibition may produce a clinically significant therapeutic effect in DIPG.

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 multicenter, open-label, 3 + 3 dose-escalation and dose-expansion clinical trial (NCT03416530) 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 newly diagnosed DIPG patients to determine the RP2D for ONC201 in combination with radiation (arm B). Dedicated assessment of intratumoral ONC201 concentrations in midline gliomas patients (arm C) and the effects of ONC201 in H3K27M DNA levels in circulating CSF (arm D) are currently enrolling patients. ONC201 as a single agent in patients with progressive H3K27M mutant tumors following irradiation (excluding DIPG/spinal cord tumors) was recently opened (arm F). Once the RP2D is confirmed, there is a dose-expansion cohort to confirm the safety, radiographic efficacy and survival with ONC201. The primary endpoints of arms A, B, and E have been established with the RP2D of 625mg scaled by body weight as a capsule or liquid formulation administered alone or in combination with radiation without incidence of dose-limiting toxicity.

DIPG-53. CHARACTERIZING THE ROLE OF PPM1D MUTATIONS IN THE PATHOGENESIS OF DIFFUSE INTRINSIC PONTINE GLIOMAS (DIPGS)

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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 and identify its associated therapeutic vulnerabilities. METHODS: We have developed multiple in vitro and in vivo models of *PPM1D*-mutant DIPGs and applied quantitative proteomic and functional genomic approaches to identify pathways altered by *PPM1Dtr* and associated dependencies. RESULTS: *PPM1D* mutations are clonal events that are anti-correlated to *TP53* mutations. 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 mutant *H3F3A* and *PDGFRA*. *PPM1Dtr* 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 *PPM1Dtr*. Furthermore, preliminary analysis of genome-wide loss-of-function CRISPR/Cas9 screens in isogenic GFP and *PPM1Dtr* overexpressing mouse neural stem cells reveal differential dependency on DNA-damage response genes in the *PPM1Dtr* overexpressing cells. Consistent with *PPM1D*'s role in stabilizing *MDM2*, *PPM1D*-mutant DIPG models are sensitive to a panel of *MDM2* inhibitors (Nutlin-3a, RG7388, and AMG232). CONCLUSION: Our study shows that *PPM1Dtr* is both an oncogene and a dependency in *PPM1D*- mutant DIPG, and there are novel therapeutic vulnerabilities associated with PPM1D that may be exploited.

DIPG-54. A NON-INVASIVE PROGNOSTIC CIRCULATING MIRNAS SIGNATURE IN DIFFUSE INTRINSIC PONTINE GLIOMAS

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Diffuse intrinsic pontine gliomas (DIPG) are the most common brainstem tumors of childhood and represent one of the most challenging paediatric tumours to treat. A non-randomized, open label phase II pilot study was conducted at Fondazione IRCCS Istituto Nazionale Tumori (Milan) to assess the efficacy in terms of objective response rate according to the RECIST criteria of combining nimotuzumab and vinorelbine with radiation in newly-diagnosed DIPG. Serum specimens were collected at baseline. microRNA expression profiling was performed using Agilent platform and Human miRNA SureSelect 8x60K containing 2006 miRNAs annotated on miRBase19.0. Primary data analysis yielded a matrix containing 330 detectable miRNA. Association with PFS allowed us to disclose a signature of 10 miRNAs able to stratify high and low risk patients (HR=4.33, 95%CI 1.49–12.54; p=4.27E-05). To test the 10 ct-miRNA model performance, we collected an independent cohort of the same sample size (n=24) and we derived the index values and risk stratification. The distribution of index values covers a range similar to the discovery cohort. Imposing the signature threshold patients were divided in high/low risk and Kaplan-Meier curves confirmed the different PFS time for the two groups with HR=3.5 (95%CI: 1.8–8.01, p-value=0.0002) for the high-risk patients, reaching AUC=0.833. Our signature is a biomarker based on non-invasive procedures for prognosis able to enter into clinical practice. Further validation on multicenter case series is warranted.

DIPG-55. PATTERNS OF CEREBROSPINAL FLUID DIVERSION AND SURVIVAL IN CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA: A REPORT FROM THE INTERNATIONAL DIPG REGISTRY

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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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