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INTRODUCTION: The role of diagnostic biopsy in diffuse intrinsic pontine glioma (DIPG) remains in question. Distinguishing radiographically between DIPG and other pontine tumors with more favorable prognosis and different therapy is critically important. METHODS: Cases submitted to the International DIPG registry with histopathologic data were analyzed. Central imaging review was performed by two neuro-radiologists; all cases with imaging features or histopathology suggestive of alternative diagnoses were re-reviewed. Imaging features suggestive of alternative diagnoses included non-pontine origin, <50% pontine involvement (without typical DIPG pattern on follow-up), focally exophytic morphology, sharply-defined margins, or marked diffusion restriction throughout. RESULTS: Among 297 patients with pathology from biopsy and/or autopsy available, 27 (9%) had histologic diagnoses not consistent with DIPG, commonly embryonal tumors (n=9) and pilocytic astrocytomas (n=11). 163 patients had diagnostic MRI available for central neuroimaging review. Among 81 patients classified as characteristic of DIPG, 80 (99%) had histopathology consistent with DIPG (diffuse midline glioma, H3K27M-mutant, glioblastoma, anaplastic astrocytoma, diffuse astrocytoma). Among 63 patients classified as likely DIPG, but with unusual imaging features, 59 (94%) had histopathology consistent with DIPG. 19 patients had imaging features suggestive of another diagnosis, including 13 with non-pontine tumor origin; the remaining 6 all had histopathology not consistent with DIPG. Association between central imaging review and histopathology was significant (p<0.001). CONCLUSIONS: The important role and accuracy of central neuroimaging review in diagnosing or excluding DIPG is demonstrated. In patients with pontine tumors for which DIPG is felt unlikely radiographically, biopsy may be considered to guide diagnosis and treatment.

DIPG-47. HISTONE MUTATIONS ENHANCE RAS MEDIATED ERK5 GROWTH SIGNALING IN DIFFUSE MIDLINE GLIOMAS

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Diffuse midline gliomas (DMGs) are incurable brain tumors with an aggressive onset. Apart from irradiation, there are currently no effective therapies available for patients with DMG, who have a median survival time of less than one year. Most DMG cells harbor mutations in genes encoding histone H3 (H3K27M) proteins, resulting in a global reduction of H3K27 trimethylation and activation of oncogenic signaling pathways. Here we show that the H3K27M mutations contribute to RAS pathway signaling, which is augmented by additional RAS activators including PDGFRA. H3K27M mutation led to increased expression of receptor tyrosine kinases (RTK). A RAS pathway functional screen identified ERK5, but not ERK1/2, as a RAS pathway effector important for DMG growth. Suppression of ERK5 decreased DMG cell proliferation and induced apoptosis *in vitro* and *in vivo*. In addition, depletion or inhibition of ERK5 significantly increased survival of mice intracranially engrafted with DMG cells. Mechanistically, ERK5 directly stabilized the proto-oncogene MYC at the protein level. Additionally, persistent ERK5 depletion does not result in complete growth inhibition and therefore we set out to determine potential adaptation or resistance mechanisms in response to ERK5 loss. Using RNA-sequencing and Immunoprecipitation (IP) mass spectrometry (IP-MS), we have identified several positive and negative feedbacks involved in ERK5 that are also targetable. These findings identify the H3K27M mutation as an enhancer of RAS activation in DMG with ERK5 and ERK5 regulated networks immediately actionable pathways.

DIPG-49. BRAINSTEM AND PONTINE VOLUMETRIC ANALYSIS AS A SURROGATE MEASURE OF LOCAL DISEASE CONTROL IN CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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INTRODUCTION: Response-assessment in pediatric neuro-oncology (RAPNO) criteria designed to describe treatment outcomes are poorly implemented in diffuse intrinsic pontine glioma (DIPG), due to inter-observer variability in measurement of tumor volume, lack of tumor enhancement, and undefined relationships between radiographic parameters and survival. Given these issues, this study assessed whether anatomically defined brainstem and pontine volumes can serve as surrogate measures of local disease burden and response to therapy in DIPG. METHODS: Thirty-two consecutive patients with newly diagnosed DIPG were treated with standard definitive radiation therapy (RT) between 2010 and 2016 at a single institution. MRI brain scans throughout treatment course were analyzed using iPlan® Flow software (Brainlab AG, Munich, Germany). Semi-automated 3D measurements of the brainstem and pons were calculated using a built-in knowledge-based segmentation approach and manually adjusted. RESULTS: Mean age at diagnosis was 6.5+/-0.5 years (range 2–12 years). Median follow up time was 317 days. Average brainstem volume at diagnosis (Vdiag) was 52.7+/-2.1mL with subsequent decrease at first post-RT MRI to 41.4+/-2.0mL ($p < 0.0001$). By time of last follow up, brainstem volume increased to 51.9+/-3.3, no longer significantly different as compared to Vdiag ($p=0.61$). The same relationships were found for pontine volume. CONCLUSIONS: Volumetric changes in the brainstem and pons occur in response to treatment and correlate with local disease burden and response to therapy. This surrogate may be a useful standardized measure in ongoing and future trials involving localized delivery of therapeutics in DIPG that require evaluation of local-regional disease control in addition to survival.

DIPG-50. A NOVEL ORTHOTOPIC PATIENT-DERIVED XENOGRAFT MODEL OF RADIATION-INDUCED GLIOMA

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Diffuse midline glioma (DMG) can arise as a primary tumour but also as a consequence of radiation therapy (RT) in survivors of other paediatric brain tumours. Radiation-associated gliomas are molecularly distinct from primary gliomas and have poorer overall survival. We report a case of radiation-associated DMG following treatment for medulloblastoma, and the development of a matched patient-derived xenograft (PDX) model. A four-year-old boy diagnosed with medulloblastoma was treated with surgical resection, RT and chemotherapy (COG:CCG-99701-Arm B). Eleven years post-diagnosis, the patient relapsed with radiation-associated DMG, participated in a Phase I clinical trial (COG:ACNS0927), and passed away eight months later. Tumour tissue collected at autopsy was intracranially implanted into immunodeficient mice and serially transplanted *in vivo*. Immunohistochemistry demonstrated both the primary DMG and PDXs expressed PDGFR-alpha and PTEN, were H3K27me3-positive, and had undetectable levels of p53. Sequencing revealed an activating mutation in PI3-kinase (H1047L) and variants of unknown significance in *GRK4*, *FLG*, *BAZ2A*, and *CRTC3*. DNA methylation array of the PDX demonstrated 1p loss, which is not typically associated with primary DMG, and broad deletion within 9p including *CDKN2A/B*, *MTAP* and multiple interferon genes. The methylation profile did not significantly classify with other tumours in the Molecular Neuropathology database (molecularneuropathology.org/mnp). We describe the first reported PDX model of radiation-associated DMG following medulloblastoma, which recapitulates the patient disease and is molecularly distinct from primary DMG. Interrogation of this model through RNA and whole genome sequencing presents a valuable opportunity to better understand and identify novel therapeutic vulnerabilities against this currently incurable disease.

DIPG-51. ACVR1 MUTATIONS PROMOTE TUMOR GROWTH IN MODELS OF DIFFUSE INTRINSIC PONTINE GLIOMA

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Mutations in the gene encoding activin A receptor type 1 (*ACVR1*) are found in approximately 25% of diffuse intrinsic pontine gliomas

(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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