predicted drug sensitivities with distinct groups of tumors predicted to respond to proteasome inhibitors, Thiotepa or Volasertib all of which have early evidence in treating gliomas. We will refine this analysis in a multi-institutional study of >100 patient gene expression profiles to define MR signatures driving known biological/molecular disease subtypes, use DIPG cell lines recapitulating common MR architectures to optimize therapy prioritization, and validate our findings *in vivo*.

DIPG-41. DISSECTING THE MECHANISTIC BASIS FOR *ACVR1* AND *PIK3CA* MUTATION CO-OCCURRENCE IN DIFFUSE MIDLINE GLIOMAS USING GENETICALLY ENGINEERED MOUSE MODELS

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Diffuse midline gliomas (DMGs) are aggressive childhood brain tumors with a dismal prognosis. Most of these tumors carry K27M mutations in histone H3-encoding genes, particularly *H3F3A* and *HIST1H3B*. In addition, activating mutations in *ACVR1* and *PIK3CA* co-occur in a subset of DMGs. To understand how these lesions drive the development of DMGs, we generated genetically engineered mouse models in which *Acvr1G328V*, *Hist1h3bK27M*, and *Pik3caH1047R* are targeted to the OLIG2-expressing cell lineage. Animals carrying *Acvr1G328V* and *Pik3caH1047R*, with ("AHPO") or without ("APO") *Hist1h3bK27M*, developed high-grade diffuse gliomas involving midline and forebrain regions. Neither *Acvr1G328V* nor *Pik3caH1047R* drove tumorigenesis by themselves, but *Acvr1G328V* was sufficient to cause oligodendroglial differentiation arrest, pointing to a role in the earliest stages of gliomas formation. Transcriptomic analyses of AHPO and APO tumors indicated a predominantly proneural and oligodendrocyte precursor-like gene expression signature, consistent with the corresponding human pathology. Genes encoding transcription factors (TFs) with dual roles in controlling glial and neuronal differentiation were upregulated in tumors. Some of these genes were mildly induced by *Acvr1G328V* alone. Functional experiments using CRISPR/Cas9-mediated gene editing in patient-derived cell lines confirmed a role for some of these TFs in controlling DMG cell fitness. Overall, our results suggest that *Pik3caH1047R* consolidates *Acvr1G328V*-induced glial differentiation arrest to drive DMG development and progression.

DIPG-42. TOWARD MULTIMODALITY THERAPY FOR DIPG/DMG: DEVELOPMENT AND INVESTIGATION OF CRANIOSPINAL IRRADIATION AND CONVECTION-ENHANCED DELIVERY PDX MODELS

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BACKGROUND: Diffuse intrinsic pontine glioma (DIPG) and diffuse midline glioma (DMG) are metastatic diseases, as demonstrated by early convection-enhanced delivery (CED) clinical trials in which prolonged local tumor control can sometimes be achieved, but fatal disseminated disease then develops. We hypothesize that improvements in treatment of both focal disease and the entire neuraxis are necessary for long-term survival, and patient-derived xenograft (PDX) models can help advance these efforts. METHODS: We used a BT245 murine orthotopic DIPG PDX model for this work. We developed a protocol and specialized platform to deliver craniospinal irradiation (CSI) with a pontine boost. We separately compared intratumoral drug concentration by CED and intraperitoneal delivery. In our CED model, mice receive gemcitabine 60 ug x1 in 15 ul at 0.5 ul/minute through a stepped catheter design with silica tubing extending 2mm beyond a 27G needle. RESULTS: Mice receiving CSI (4 Gy x2d) plus boost (4 Gy x2d) showed minimal spinal and brain leptomeningeal metastatic disease by bioluminescence, MRI, and pathology compared to mice receiving radiation to the pons only (4 Gy x4d) or no radiation. CED achieved an intratumoral gemcitabine concentration 50-fold greater than intraperitoneal dosing when controlled for dose. CONCLUSIONS: In a DIPG PDX model, CSI+boost minimizes tumor dissemination compared to focal radiation, and CED achieves clinically significant improvements in intratumoral chemotherapy concentration compared to systemic delivery. Adding these modalities to current treatment could improve both focal and metastatic tumor control, leading to meaningful improvements in survival.

DIPG-43. CAN WE REPROGRAM DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)? EXPLORING THE ROLE OF DISTALLESS/DLX HOMEOBOX GENE REGULATION OF OLIGODENDROGLIAL PROGENITOR CELLS (OPC) IN THE DEVELOPING VERTEBRATE NERVOUS SYSTEM

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BACKGROUND: The identification of H3.3/H3.1K27M in most DIPG has changed our understanding of this disease. H3K27M mutations usually demonstrate global loss of H3K27 trimethylation (me^3) with gain of H3K27 acetylation (ac). Single cell RNAseq has identified the putative cell of origin as oligodendroglial progenitor cells (OPC). The *distalless* gene family is necessary for the differentiation and tangential migration of committed neural progenitors to become GABAergic interneurons. *Dlx1/Dlx2* double knockout (DKO) cells from the ganglionic eminences (GE) transplanted into a wild-type environment become oligodendrocytes. RESULTS: We identified DLX2 occupancy of early (*Olig2, Nkx2.2*) and late (*Myt1, Plp1*) genes required for OPC differentiation *in vivo* and confirmed direct DLX2 protein-promoter DNA binding *in vitro*. Co-expression of *Dlx2* with target sequences reduced reporter gene expression *in vitro*. There was increased expression of OLIG2, NKX2.2 and PLP-1 expression *in vivo*, consistent with de-repression in the absence of *Dlx1/Dlx2* function. Transient overexpression of a *Dlx2*-GFP construct into murine DIPG cells from a GEMM that develops DIPG resulted in significant increases in expression of *Gad* isoforms with concomitant decreases in *Olig2* and *Nkx2.2*. *Dlx2*-transfected mDIPG cells also demonstrated reduced migration, invasion and colony formation *in vitro*. Of significance, there was global restoration of $H3K27me^3$ with corresponding loss of H3K27ac expression in transfected cells compared to controls. CONCLUSIONS: DLX2 promotes GABAergic differentiation and migration while concomitantly repressing OPC differentiation *in vivo*. Developmental reprogramming of mDIPG cells by DLX2 demonstrates the potential role for directed differentiation strategies towards improving patient outcomes for this devastating pediatric cancer.

DIPG-44. A GAIN OF FUNCTION EZH2 MUTATION DELAYS DIFFUSE INTRINSIC PONTINE GLIOMA PROGRESSION

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BACKGROUND: Diffuse Intrinsic Pontine Glioma (DIPG) remains an incurable pediatric brain cancer. The oncohistone H3K27M implicated in 80% of the cases, is also predicted to target Enhancer of Zeste Homolog 2 (Ezh2), the catalytic component of the Polycomb Repressor Complex 2 (PRC2). There are no reported mutations of Ezh2 and its function in DIPG is not fully determined. This work aims to address the role of Ezh2 in DIPG. METHODS: Brainstem tumors were established by intracranial injections of *Nestin*;Tv-a; $Ezh2^{Y641F/+}$ (NTv-a; $Ezh2^{Y641F/+}$) neonatal pups using Replication Competent Avian Sarcoma leucosis virus long terminal repeat with splice acceptor (RCAS) viruses, expressing PDGF-B, p53 shRNA, and RCAS-CRE/Y. Immunohistochemical staining for Ki-67 and H3K27me3 were performed on the Discovery ULTRA (Ventana). RESULTS: Ezh2 overexpression ($Ezh2^{Y641F/+}$, RCAS CRE) conferred a survival advantage of approximately 10 days (n=20 mice/group, $p<0.001$). H3K27me3 levels were significantly upregulated in RCAS CRE group (50% vs 20% in RCAS Y, n=4 tumors/group, $p<0.03$), with a concomitant lower Ki-67 staining (30% vs. 55% in RCAS Y, n=3 tumors/group, $p<0.05$). Interestingly, pathological review categorized more RCAS-CRE tumors as 'atypical'. RNA-sequencing of virus-infected neural precursor cells revealed a suppression of inflammatory/interferon gene signature in the Ezh2 overexpression group. CONCLUSIONS AND FUTURE DIRECTIONS: Enhanced Ezh2 activity appears to delay DIPG pathogenesis. Ongoing work aims to highlight the contribution of differentially expressed gene signatures that contribute to this phenotype.

DIPG-46. NON-DIPG PATIENTS ENROLLED IN THE INTERNATIONAL DIPG REGISTRY: HISTOPATHOLOGIC EVALUATION OF CENTRAL NEURO-IMAGING REVIEW

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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