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

Annette Wu, Tak Mak, and Jerome Fortin; Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

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

Aaron J Knox[1], Ahmed Gilani[1], Benjamin van Court[1], Ayman Oweida[1], Patrick Flannery[1], John DeSisto[1], Rakeb Lemma[1], Hannah Chatwin[1], Fabia Gamboni[1], Benjamin Brown[1], Natalie Serkova[1], Rajeev Vibhakar[1], Kathleen Dorris[1], Michael Wempe[2], Julie A Reisz[1], Sana D Karam[1], and Adam L Green[1]; [1]The Morgan Adams Foundation Pediatric Brain Tumor Research Program, University of Colorado School of Medicine/Children's Hospital Colorado, Aurora, CO, USA, [2]University of Colorado School of Pharmacy and Pharmaceutical Sciences, Aurora, CO, USA

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

Mikaela Nevin[1], Janine Gallego[1], Xiaohua Song[1], Qiang Jiang[1], Alan Underhill[1], Roseline Godbout[1], Oren Becher[2], and David Eisenstat[1]; [1]University of Alberta, Edmonton, AB, Canada, [2]Northwestern University, Chicago, IL, USA

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

Swati Dhar[1,2], Samantha Gadd[1], Daniel Brat[2], and Oren Becher[1,2]; [1]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA, [2]Northwestern University, Chicago, IL, USA

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

Margot A. Lazow[1], Christine Fuller[1], Adam Lane[1], Mariko D. DeWire-Schottmiller[1], Pratiti Bandopadhayay[2], Ute Bartels[3], Eric Bouffet[3], Sylvia Cheng[4], Kenneth J. Cohen[5], Tabitha M. Cooney[6], Scott L. Coven[7], Hetal Dholaria[8], Blanca Diez[9], Kathleen Dorris[10], Motasem El-Ayadi[11], Ayman El-Sheikh[12], Paul G. Fisher[13], Mercedes Garcia Lombardi[14], Robert J. Greiner[15], Stewart Goldman[16], Nicholas Gottardo[8], Sridharan Gururangan[17], Jordan R. Hansford[18], Tim Hassall[19], Cynthia Hawkins[3], Lindsay Kilburn[20], Carl J. Koschmann[21], Sarah E. Leary[22], Jie Ma[23], Jane E. Minturn[24], Michelle Monje-Deisseroth[13], Roger J. Packer[20], Yvan Samson[25], Eric S. Sandler[26], Gustavo Sevlever[9], Christopher Tinkle[27], Karen Tsui[28], Lars M. Wagner[29], Mohamed Zaghloul[11], David S. Ziegler[30], Brooklyn Chaney[1], Katie Black[1], Anthony Asher[1], Rachid Drissi[1], Maryam Fouladi[1], Blaise V. Jones[1], and James L. Leach[1]; [1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [2]Boston Children's Hospital, Boston, MA, USA, [3]Hospital for Sick Children, Toronto, ON, Canada, [4]BC Children's Hospital, Vancouver, BC, Canada, [5]Johns Hopkins, Baltimore, MD, USA, [6]UCSF Benioff Children's Hospital Oakland, Oakland, CA, USA, [7]Riley Children's Health, Indianapolis, IN,