predicted drug sensitivities with distinct groups of tumors predicted to re-pond 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 pri-oritization, and validate our findings in vivo.

DIPG-41. DISSECTING THE MECHANISTIC BASIS FOR ACVR1 AND PTK2CA MUTATION CO-OCCURRENCE IN DIFFUSE MIDLIME GLIOMAS USING GENETICALLY ENGINEERED MOUSE MODELS

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Diffuse midline gliomas (DMGs) are aggressive childhood brain tumors with poor survival. Most of these tumors carry K27M mutations in their H3-encoding genes, particularly H3F3A and HIST1H3B. In addition, activating mutations in ACVR1 and PTK2CA co-occur in a subset of DMGs. To understand how these lesions drive the development of DMGs, we generated genetically engineered mice harboring PTK2CA and Ptk2caH1047R mutations are targeted to the OLIG2-expressing cell lineage. Animals carrying AcertG128V and PtkcaH1047R, with (“APHO”) or without (“APO”) H1attb3K2M7, developed high-grade diffuse gliomas involving mid-line and forebrain regions. Neither AcertG128V nor Ptk2caH1047R drove tumorigenesis by themselves, but AcertG128V was sufficient to cause oligo-dendroglial differentiation arrest, pointing to a role in the earlier stages of gliomas formation. Transcriptomic analyses of APHO and APO tumors indi-cate a predilection for the prerenal and oligodendrocyte precursor-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 AcertG128V 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 PtkcaH1047R consolidates AcertG128V-induced glial differentiation arrest to drive DMG development and progression.

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

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BACKGROUND: Diffuse intrinsic pontine glioma (DIPG) is 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). Ezh2, 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-Fv2; Ezh2F+/-; NTv-A (NTv-A, Ezh2F+/-) neonatal pups using Replication Competent Avian Sarcoma leucosis virus long terminal repeat with splice acceptor (RCAS) viruses, expressing PDGF-B, p53 shRNA, and CRAC/CREY. Immunohistochemical staining for Ki-67 and H3K27me3 was performed on the DIPG ULTRA (Ventana). RESULTS: Ezh2 overexpression (Ezh2; Fv2; NTv-A) 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. 35% in RCAS Y, n=3 tumors/group, p<0.03). Interestingly, pathologica-ly reviewed tissue 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, CONCLU-SIONS: BET FUTURE DIRECTIONS: Enhanced Ezh2 activity results in delay DIPG pathogenesis. Ongoing work aims to highlight the contribution of differentially expressed gene signatures that contribute to this phenotype.

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

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BACKGROUND: The identification of H3.3/F3.H1.K27M in most DIPG has changed our understanding of this disease. H3K27M mutations usu-ally demonstrate global loss of H3K27 trimethylation (me3) with gain of H3K27 acetylation (ac). Single cell RNASeq has identified the putative cell of origin oligodendrogliomatous precursor cells (OPC). The OPC population is necessary for the differentiation and tangential migration of committed neural progenitors to become GABAergic interneurons. Delx1/Delx2 double knockout (DKO) cells from the ganglionic eminences (GE) transplanted into immature-oligodendroglial regeneration (RE) model. 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 Delx2 with target sequences reduced reporter gene expression in vitro. This was increased expression of OLIG2, Nkx2.2 and PLP-1 expression in vivo, consistent with de-repression in the absence of Delx1/Delx2 function. Transient over-expression of a Delx2-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. Delx2-transfected mDIPG cells also demonstrated reduced migration, invasion and colony for-mation in vitro. Of significance, there was global restoration of H3K27me3 with corresponding loss of H3K27ac expression in transducted cells com-pared to DIPG (DELX2) and DIPG+transduced Enhanced Ezh2 activity, accounting for 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 DIPG IN A DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) MOUSE MODEL

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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). Ezh2, 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-Fv2; Ezh2F+/-; NTv-A (NTv-A, Ezh2F+/-) neonatal pups using Replication Competent Avian Sarcoma leucosis virus long terminal repeat with splice acceptor (RCAS) viruses, expressing PDGF-B, p53 shRNA, and CRAC/CREY. Immunohistochemical staining for Ki-67 and H3K27me3 was performed on the DIPG ULTRA (Ventana). RESULTS: Ezh2 overexpression (Ezh2; Fv2; NTv-A) 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. 35% in RCAS Y, n=3 tumors/group, p<0.03). Interestingly, pathologica-ly reviewed tissue 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, CONCLU-SIONS: BET FUTURE DIRECTIONS: Enhanced Ezh2 activity results in delay DIPG pathogenesis. Ongoing work aims to highlight the contribution of differentially expressed gene signatures that contribute to this phenotype.

DIPG-45. NON-DIPG PATIENTS ENROLLED IN THE INTERNATIONAL DIPG REGISTRY: HISTOPATHOLOGIC EVALUATION OF CENTRAL NEURO-IMAGING REVIEW

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DIPG-46. HOMEOBOX GENE REGULATION OF OLIGODENDROGLIAL PROGENITOR CELLS (OPC) IN THE DEVELOPING VERTEBRATE NERVOUS SYSTEM

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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 5-year-old boy diagnosed with medulloblastoma, with tumour progression after four cycles of chemotherapy and definitive radiation therapy (RT) between 2010 and 2016 at a single institution, was treated with an extended field of radiation and then palliative chemotherapy. Radiation-associated gliomas are molecularly distinct from primary DMG. In vivo molecular modelling of radiation-associated DMG following treatment for medulloblastoma, which recapitulates the patient disease and is molecularly distinct from primary DMG. Interrogation of this model through in vivo immunohistochemistry demonstrated both the primary DMG and PDXs expressed PDGFRa- and PTEN, were H3K27me3-positive, and had undetectable levels of p53. The tumour DNA sequence collected at autopsy was intraradion and implanted into immunodeficient mice and serially transplanted in vivo. Immunohistochemistry demonstrated both the primary DMG and PDXs expressed PDGFRa- and PTEN, were H3K27me3-positive, and had undetectable levels of p53. The tumour DNA sequence collected at autopsy was intraradion and implanted into immunodeficient mice and serially transplanted in vivo. Immunohistochemistry demonstrated both the primary DMG and PDXs expressed PDGFRa- and PTEN, were H3K27me3-positive, and had undetectable levels of p53. The tumour DNA sequence collected at autopsy was intraradion and implanted into immunodeficient mice and serially transplanted in vivo. Immunohistochemistry demonstrated both the primary DMG and PDXs expressed PDGFRa- and PTEN, were H3K27me3-positive, and had undetectable levels of p53. The tumour DNA sequence collected at autopsy was intraradion and implanted into immunodeficient mice and serially transplanted in vivo.