zebrafish as an accurate investigative toxicology model to assess acute toxicity of molecules in preclinical studies. Conclusions: By testing a wide range of drugs, targeting different pathways on DMG cells and in different in vivo systems we identified promising drug candidates for clinical management of children diagnosed with DMG.

HGG-24. PRECLINICAL EFFICACY OF THE BRAIN PENETRANT CYCLIN-DEPENDENT KINASE INHIBITOR ZOTIRACICLIB IN PEDIATRIC DIFFUSE MIDLINE GLIOMAS

Timothy Mueller[1], Sridevi Yadavilli[2], Sandra Laternser[1], Chiara Cianciolo[1], Justyna Przystal[1], Erin Bonner[1,2], Matt C Biery[3], Nicholas A Vitanza[3,4], Michael Grotzer[1], Sabine Mueller[1,5], and Javad Nazarian[1,2]; [1]DMG Research Center Zurich, Oncology Department, University Children's Hospital Zurich, Zurich, Switzerland, [2]Center for Genetic Medicine Research, Children's National Hospital, Washington, DC, USA, [3]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [4]Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of Washington, Seattle Children's Hospital, Seattle, WA, USA, [5]UCSF Department of Neurology, Neurosurgery and Pediatrics, San Francisco, CA, USA

Children diagnosed with diffuse midline gliomas (DMG), including diffuse intrinsic pontine glioma (DIPG), have extremely poor outcomes with a median overall survival of 9–12 months from initial diagnosis. Standard-of-care is limited to focal radiation therapy, given the paucity of effective targeted therapies for DMG. To identify effective drugs for treatment of children diagnosed with DMG, we investigated the brain-penetrating multi cyclin-dependent kinase inhibitor Zotiraciclib (ZTR/TG02). ZTR has demonstrated encouraging response rates and a benign safety profile in phase 1 trials of adults with high-grade glioma. It is thought to achieve its anticancer activity mainly by transcription disruption, a previously described vulnerability of DMGs, by inhibiting multiple cyclin-dependent kinases 9 and 7 (CDK9, 7). We found that ZTR robustly reduces viability of different patient derived DMG cells in a dose-dependent manner, with a median IC_{50} of 201 nM across eight tested cell lines (range 11–1258 nM, 72 hrs). Consistently, we observed loss of RNA polymerase II phosphorylation after 24 hours of treatment, indicating effective CDK9 inhibition at low drug concentrations and after short incubation time. This effect was followed by depletion of short-lived proteins including MYC and the anti-apoptotic factor MCL-1. Putative biomarkers of response and resistance were identified in silico using DepMap data analysis. To assess the safety profile of ZTR, we exposed our zebrafish model to various drug concentrations and found the drug to be safe at IC_{50} molarity. Ongoing *in vitro* and *in vivo* studies evaluating the efficacy of ZTR in combination with promising combination therapies for more effective treatment of children with DMG are also underway.

HGG-25. PRMT5 PROMOTES TUMOR GROWTH BY MAINTAINING STEMNESS OF PEDIATRIC HIGH-GRADE GLIOMA CELLS

John DeSisto[1], Aaron Knox[1], Hannah Chatwin[1], Ilango Balakrishnan[1], Sujatha Venkataraman[1], Rajeev Vibhakar[1,2], and Adam Green[1,2]; [1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [2]Children's Hospital Colorado, Aurora, CO, USA

Background: Pediatric high-grade gliomas (pHGG) are aggressive tumors that together constitute the most common cause of childhood cancer mortality. Tumor stem cells that drive proliferation of pHGG resist chemotherapy and radiation, complicating treatment. The arginine methyltransferase PRMT5 maintains self-renewal in neural stem cells through epigenetic modifications. We hypothesized that PRMT5, which we identified as a potential driver of diffuse midline glioma (DMG) through an shRNA screen, plays a similar role in pHGG. Methods: Using lentiviral delivery of shRNA, we knocked down (KD) PRMT5 in cortical pHGG and DMG cell lines and performed phenotypic, mechanistic and self-renewal assays. We irradiated PRMT5 KD and control cells to study sensitization. We orthotopically injected mice with PRMT5 KD pHGG cells, and with DMG cells in which PRMT5 was knocked out (KO) using CRISPR-Cas. Results: In cellular models of cortical pHGG and DMG, PRMT5 KD significantly reduced proliferation, inhibited cell cycle progression, increased apoptosis resistance, and decreased self-renewing cell frequency. A relative shift of PRMT5 from the cytoplasm to the nucleus accompanied differentiation induced by PRMT5 KD. Epigenetic changes accompanying PRMT5 KD included increased H3K27me3, a global transcription inhibitor, and decreased H3K27M expression in DMG. PRMT5 KD sensitized pHGG cells to radiation, increasing cell death 17–30%. PRMT5 KD/KO significantly increased survival in mice and decreased tumor aggressiveness and proliferation, but mice still died of tumor-related effects. Conclusions: PRMT5 maintains self-renewal and drives proliferation in preclinical pHGG models. In cellular and *in vivo* models, PRMT5 KD/KO produces epigenetic changes, including increased H3K27me3 levels and diminished H3K27M, that may reduce proliferation and self-renewal. Future work includes elucidation of the mechanisms by which PRMT5 produces the observed changes. Because PRMT5 KD/KO does not eliminate tumor growth, we plan to further study combining PRMT5 KD/KO and clinical-grade small molecule PRMT5 inhibitors with radiation and chemotherapeutic agents.

HGG-26. SINGLE-CELL RNA-SEQ OF PEDIATRIC HIGH-GRADE GLIOMAS IDENTIFIES COMMON ONCOGENIC PROCESSES AMONG DISTINCT TUMOR HISTOLOGIES

John DeSisto[1], Andrew Donson[1], Rui Fu[1], Bridget Sanford[1], Kent Riemondy[1], and Adam Green[1,2]; [1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [2]Children's Hospital Colorado, Aurora, CO, USA

Background: Pediatric high-grade glioma (PHGG) is a deadly childhood brain tumor that responds poorly to treatment. PHGG comprises two major subtypes: cortical tumors with wild-type H3K27 and diffuse midline gliomas (DMG) that occur in the midline and have characteristic H3K27M mutations. Cortical PHGG is heterogeneous with multiple molecular subtypes. In order to identify underlying commonalities in cortical PHGG that might lead to better treatment modalities, we performed molecular profiling, including single-cell RNA-Seq (scRNA-Seq), on PHGG samples from Children's Hospital Colorado. Methods: Nineteen cortical PHGG tumor samples, one DMG and one normal margin sample obtained at biopsy were disaggregated to isolate viable cells. Fifteen were glioblastomas (GBM), including five with epithelioid and/or giant cell features and five radiation-induced glioblastomas (RIG). There were also four non-GBM PHGG. We performed scRNA-Seq using 10X Genomics v.3 library preparation to enable capture of infiltrating immune cells. We also performed bulk RNA-Seq and DNA methylation profiling. Results: After eliminating patient-specific and cell-cycle effects, RIG, epithelioid GBM, and other GBM each formed identifiable subgroups in bulk RNA-Seq and scRNA-Seq datasets. In the scRNA-Seq data, clusters with cells from multiple tumor samples included a PDGFRA-positive population expressing oligodendrocyte progenitor markers, astrocytic, mesenchymal and stemlike populations, macrophage/monocyte immune cells, and a smaller T-cell population. Analyses of DNA methylation data showed PDGFRA and CDK4 amplification and CDKN2A deletion are common alterations among PHGG. Inferred copy number variation analysis of the single-cell data confirmed that individual tumors include populations that both include and lack the molecular alterations identified in the methylation data. RNA velocity studies to define tumor cells of origin and further analyses of the immune cell populations are underway. Conclusions: Single-cell analysis of PHGG confirms a large degree of tumor heterogeneity but also shows that PHGG have stemlike, mesenchymal and immune cell populations with common characteristics.

HGG-27. HNRNPA1 SPLICED VARIANT SENSITIZATION EFFECT DISCLOSED IN GLIOMA CELLS

Ajay Yadav Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India

Glioblastoma is aggressive brain tumor. Glioma heterogeneity builds in hypoxic condition due to its intrinsic high apoptosis rate cause to develop a high selection clonal pressure. HnRNPA1 plays a key role in developing glycolytic tumor, shows its high expression exclusively in hypoxic glioma cells. Recently we observed one more spliced variant of hnRNPA1, encoding higher isoform, exclusively abundant in resistant glioma cell lines. Widely around the scientific community HnRNPA1 splice factor family protein was found distinctly regulating resistant glioma phenotype. To support our hypothesis, methodology we perform includes various apoptosis assays to critically understand hnRNPA1 spliced variant dependent pathway in Temozolomide resistant U87 glioma cells. Proteomic based apoptotic array and angiogenic array enable us to visualize selective knock down of hnRNPA1 has dominant role in promoting apoptotic cascade. Additionally, flow cytometry base annexin V-PI staining technique to understand early and late apoptosis was measured in selective hnRNPA1 spliced variant knockdown cells in presence or absence of PI3 kinase inhibitor wortmannin (5 micro molar). Results showed hnRNPA1 higher isoform knock down promotes more apoptosis compare to lower isoform. Interestingly, overexpression of HnRNPA1 higher isoform or lower isoform alone doesn't promote apoptosis, however is prominently higher apoptosis in Bortezomib treated U87 glioma cells. These both isoforms are presently majorly in gliomas, but somehow for long was not recognized. Conclusion is to explore more related novel finding or therapeutic strategy to target higher isoform of hnRNPA1, using invivo mouse xenograft model.