survival of <1 year. The critical location in the brainstem and the often intact blood-brain barrier (BBB) pose significant challenges in the treatment of DIPG. The objective of this study was to demonstrate the potential for focused ultrasound-induced BBB disruption (FUS-BBBD) to improve DIPG treatment by enhancing the safe and efficient delivery of drugs. A genetically engineered mouse model of DIPG was generated using the RCAS (replication-competent avian sarcoma-leucosis virus long-terminal repeat with splice acceptor)/tumor virus A modeling system. A magnetic resonance-guided FUS (MRgFUS) system was used to induce BBB disruption in these mice with the FUS targeted at the center of the tumor. Two radiolabeled agents with different sizes were used to evaluate the delivery efficiency of the FUS-BBBD technique in DIPG mice: a small-molecular radiotracer, ^{68}Ga-DOTA-ECL1i, and a radiolabeled nanoparticle, ^{64}Cu-labeled copper nanoparticles (^{64}Cu-CuNCs, ~ 5 nm in diameter). ^{68}Ga-DOTA-ECL1i (half-life ~ 1 h) and ^{64}Cu-CuNCs (half-life ~13 h) were intravenously injected into the mice after FUS sonication, and microPET/CT imaging was performed at 1 h and 24 h, respectively, to evaluate the spatial-temporal distribution of these two agents in the brain and quantify the delivery outcome. FUS treatment increased the uptake of ^{68}Ga-DOTA-ECL1i and ^{64}Cu-CuNCs to the DIPG tumor by 3.25 folds and 4.07 folds on average, respectively. These findings demonstrated, for the first time, that FUS can increase BBB permeability in a murine model of DIPG and significantly enhance the delivery of agents of different sizes into the DIPG tumor.

HGG-19. 5-AMINOLEVULINIC ACID (5-ALA)-GUIDED RESECTION OF PEDIATRIC BRAIN TUMORS

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Between tumor and normal brain, allowing a higher degree of resection, and improved patient outcomes. In recent years, several reports have emerged regarding the use of 5-ALA in other brain tumor entities, including pediatric brains tumors. Since gross total resection (GTR) of many brain tumors in children is crucial, the role of 5-ALA-guided resection requires elucidation.

Methods: A systematic literature review of EMBASE and MEDLINE/PubMed databases revealed 20 eligible publications encompassing 186 5-ALA-guided operations on pediatric brain tumors. To reduce bias, publications were revised independently by two authors. Results: 5-ALA-guided resection enabled the surgeons to identify the tumor more easily and was considered helpful mainly in cases of glioblastoma (GBM, 21/27, 78%), anaplastic ependymoma WHO grade III (10/14, 71%), and anaplastic astrocytoma (4/6, 67%). In contrast, cases of pilocytic astrocytomas (PAs) and medulloblastomas 5-ALA-guided surgery did not show consistent fluorescent signals and 5-ALA was considered helpful only in 12% and 22% of cases, respectively. Accumulation of fluorescent porphyrins seems to depend on WHO tumor grading. In case fluorescence signal was considered helpful, it was associated with a greater degree of resection. One study showed an association between visible fluoresce signal and concentration of protopophyrin IX (PPIX) concentration. A threshold of 4µg/ml was required in order to visualize the fluorescence signal. The rate of adverse events related to 5-ALA was negligible, especially new postoperative sequelae. Conclusion: 5-ALA could play a role in resection of malignant, contrast enhancing, supratentorial pediatric brain tumors. At present, we are conducting a prospective phase I-II multicenter clinical trial to evaluate side effects and feasibility of 5-ALA guided surgery.

HGG-21. MALIGNANT SYNAPTIC PLASTICITY IN PEDIATRIC HIGH-GRADE GLIOMAS

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Pediatric high-grade gliomas (pHGG) are a devastating group of diseases that urgently require novel therapeutic options. We have previously demonstrated that pHGGs directly synapse onto neurons and the subsequent tumor cell depolarization, mediated by calcium-permeable AMPA channels, promotes their proliferation. The regulatory mechanisms governing these postsynaptic connections are unknown. Here, we investigated the role of BDNF-TrkB signaling in modulating the plasticity of the malignant synapse. BDNF ligand activation of its canonical receptor, TrkB (which is encoded for by the gene *NTRK2*), has been shown to be one important modulator of synaptic regulation in the normal setting. Electrophysiological recordings of glioma cell membrane properties, in response to acute neurotransmitter stimulation, demonstrate in an inward current resembling AMPA receptor (AMPAR) mediated excitatory neurotransmission. Extracellular BDNF increases the amplitude of this glutamate-induced tumor cell depolarization and this effect is abrogated in *NTRK2* knockout glioma cells. Upon examining tumor cell excitability using in situ calcium imaging, we found that BDNF increases the intensity of glutamate-evoked calcium transients in GCaMP6s expressing glioma cells. Western blot analysis indicates the tumors AMPAR properties are altered downstream of BDNF induced TrkB activation in glioma. We find that BDNF-TrkB signaling promotes neuron-to-glioma synaptogenesis as measured by high-resolution confocal and electron microscopy in culture and tumor xenografts. Our analysis of published pHGG transcriptomic datasets, together with brain slice conditioned medium experiments in culture, indicate the tumor microenvironment as the chief source of BDNF ligand. Disruption of the BDNF-TrkB pathway in patient-derived orthotopic glioma xenograft models, both genetically and pharmacologically, results in an increased overall survival and reduced tumor proliferation rate. These findings suggest that gliomas leverage mechanisms of plasticity to modulate the excitatory channels involved in synaptic neurotransmission and they reveal the potential to target the regulatory components of glioma circuit dynamics as a therapeutic strategy for these lethal cancers.

HGG-22. EVALUATING THE REGULATION OF BLOOD-BRAIN BARRIER INTEGRITY IN DIPG MOUSE MODELS

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Diffuse intrinsic pontine gliomas (DIPGs) are considered to maintain a fairly intact blood-brain barrier (BBB) based on patient imaging tumor histology. In characterizing recently developed DIPG and HGG mouse models, we identified differences in BBB function and increased Angiopoietin1 (Angpt1) in H3 K27M DIPG models. We hypothesize that H3 K27M mutations promote the maintenance of DIPG BBB integrity through upregulation of Angpt1. To determine DIPG and HGG BBB phenotypes we performed an intergrative analysis of vascular histology and endothelial transcriptomes Ongoing studies using electroporation based DIPG mouse models are being performed examine the regulation and function of Angpt1 in DIPG BBB integrity. We have initiated studies comparing H3 K27M DIPG mouse models to H3 WT and G34R cortical HGG mouse models, demonstrating that DIPG models show minimal changes in vascular phenotype, including vessel density, branching, and diameter compared to cortical HGG models. Comparing DIPG and HGG purified endothelial transcriptomes, HGG ECs displayed enrichments of inflammatory signals and proliferation gene sets, and increased expression of tip cell identity genes. We identified Angpt1 as selectively upregulated in H3 K27M mouse models and derived cell lines. Preliminary data suggests Angpt1 supports the maintenance of BBB integrity in DIPG models. BBB phenotype differences are present in DIPG and HGG mouse models. Uncovering mutation specific mechanisms that regulate BBB function in brain tumors will be critical to advance our understanding of brain tumor pathogenesis and treatment response.

HGG-23. IN VITRO AND IN VIVO PRECLINICAL DRUG SCREENING OF PROMISING THERAPEUTICS FOR DIFFUSE MIDLINE GLIOMA (DMG)

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Introduction: Diffuse midline gliomas (DMGs) are amongst the most unforgiving pediatric brain tumors, characterized by an intrinsic resistance to therapy. Despite major advances in understanding of tumor biology, the prognosis remains exceedingly poor, and treatment options are limited. New therapeutics are being evaluated at a fast rate by different laboratories. In order to prioritize effective drug candidates for DMG treatment, we comprehensively characterized a panel of promising therapeutic agents in in vitro and in different vivo systems. Methods: We determined the sensitivity of primary DMG cell lines to a panel of small molecule inhibitors targeting known DMG targets and pathways. Dose response curves were generated for more than 20 different compounds and possible synergistic effects were investigated by SynergieFinder. In an effort to highlight potential toxicities and associated mechanisms at a large scale, we performed a preclinical toxicity evaluation in zebrafish larvae, with a slightly modified version of the official Fish Embryo Acute Toxicity (FET) test. Drug toxicity was tested by continuous exposure of zebrafish larvae to increasing concentrations of the different compounds. Survival curves, morphological analyses and behavioral tests were performed at a maximum tolerated dose (MTD). To confirm the findings obtained in zebrafish, we further performed in vivo studies in mice for promising candidates. Results: Among the tested drugs in vitro we found 10 drugs showing promising dose- dependent reduction in cell viability with IC_{50} in nM to µM range. These were further evaluated for toxicity in zebrafish. The zebrafish larvae toxicities observations strongly correlated with the findings in murine in vivo studies, reinforcing the importance of

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

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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

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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

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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

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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.