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DIPG is an aggressive and incurable childhood brain tumour for which new treatments are needed. A high throughput drug screen of 3500 pharmaceutical compounds identified anti-malarials, including quinacrine as having potent activity against DIPG neurospheres. CBL0137, a compound modelled on quinacrine, is a novel anti-cancer compound which targets Facilitates Chromatin Transcription (FACT), a chromatin remodelling complex involved in transcription, replication, and DNA repair. CBL0137 effectively crosses the blood-brain barrier and has recently completed Phase I testing in adult patients. CBL0137 induced apoptosis in DIPG neurospheres and had profound cytotoxic activity against a panel of DIPG cultures. In a DIPG orthotopic model, treatment with CBL0137 significantly improved survival. We found that treatment with CBL0137 up-regulated TP53 and increased histone H3.3 acetylation and tri-methylation in DIPG cells. We therefore examined the interaction between CBL0137 and the histone deacetylase (HDAC) inhibitor panobinostat. *In vitro* experiments showed that the two agents had profound synergistic activity against DIPG neurospheres in clonogenic assays and enhanced caspase activation and apoptosis. The FACT subunit SSRP1 was found to directly interact with H3.3K27M and treatment with CBL0137 targeted this epigenetic defect, restoring histone H3.3 trimethylation and leading to tumor cell death. Transcriptomic analysis and immunoblotting indicated that combination treatment activated signalling pathways controlled by Retinoblastoma (RB)/E2F1 and subsequently increased phosphorylation and enzymatic activity of enhancer of zeste homolog 2 (EZH2). Consistent with the *in vitro* results, the combination of CBL0137 and panobinostat significantly prolonged survival in two independent orthotopic models of DIPG, while histological analysis showed restoration of H3K27me3 and decreased Ki67 positive cells. In addition to panobinostat, CBL0137 has been found to combine synergistically *in vitro* and *in vivo* with PARP and BET inhibitors. Given these promising results, a paediatric trial of CBL0137 will open through the Children's Oncology Group with an expansion cohort for DIPG patients.

HGG-10. THE BLOOD-BRAIN BARRIER IN DIPG: INVESTIGATING REGION-SPECIFIC DIFFERENCES IN PERMEABILITY

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Diffuse Intrinsic Pontine Glioma (DIPG) is the most aggressive pediatric high-grade glioma with median survival of only 12 months from diagnosis. Current therapies are essentially palliative. The blood-brain barrier (BBB) is a major obstacle, limiting delivery of effective chemotherapeutics into the brain. We hypothesized that tumors in the brainstem region have a BBB less permeable than tumors in other brain regions. We have confirmed the presence of an intact BBB in three orthotopic models of DIPG by Evans Blue extravasation assay. Immunohistochemical staining of CD13+ pericytes and CD34+ endothelial cells in healthy mouse brain compared to orthotopic DIPG model showed higher levels of both components in brainstem compared to cortical region. Single-cell RNA sequencing experiments are currently being undertaken to investigate region-specific differences in BBB cell populations and the impact of DIPG on signaling pathways that govern permeability. To determine if tumor location impacts therapeutic outcome, we performed *in vivo* efficacy studies with DIPG orthotopically injected into cortical region or brainstem region and treated with SAHA, HDAC inhibitor, or temsirolimus, mTOR inhibitor. Temsirolimus or SAHA was ineffective at extending survival in mice injected with DIPG in the brainstem compared to control. However, temsirolimus led to a significant improvement in survival in mice injected with DIPG cells in cortical region (median survival 85 days) compared to control (median survival 69 days ($P \leq 0.01$)). This suggests that the same tumor in cortical region may respond to systemic therapy that is ineffective in the brainstem and that the intact BBB in the brainstem is a major reason for treatment failure in DIPG. In conclusion, the BBB in the brainstem and in the presence of DIPG may be altered, changing signaling pathways that affect permeability. Understanding the brainstem cerebrovasculature may potentially lead to a novel strategy to treat DIPG as well as other brain tumors.

HGG-11. LEPTOMENINGEAL DISEASE AND TUMOR DISSEMINATION ALONG CSF PATHWAYS IN A MURINE DIPG MODEL: IMPLICATIONS FOR STUDY OF THE TUMOR-CSF-EPENDYMAL MICROENVIRONMENT

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Background: Leptomeningeal disease and hydrocephalus are present in up to 30% of patients with diffuse intrinsic pontine glioma (DIPG), however there are no animal models of cerebrospinal fluid (CSF) dissemination. As the tumor-CSF-ependymal microenvironment may play an important role in tumor pathogenesis, we identified characteristics of the Nestin-tumor virus A (Nestin-Tva) genetically engineered mouse model (GEMM) that make it ideal to study the interaction of tumor cells with the CSF and its associated pathways with implications for the development of treatment approaches to address CSF dissemination in DIPG. Methods: A Nestin-TVa model of DIPG utilizing the three most common DIPG genetic alterations (H3.3K27M, PDGF-B, p53) was used for this study. All animals underwent MR imaging and a subset underwent histopathologic analysis with H&E and beta-IV tubulin. Results: Tumor dissemination within the CSF pathways (ventricles, leptomeninges) was present in 76% (25/33) of animals, with invasion of the choroid plexus, disruption of the ciliated ependyma and regional subependymal fluid accumulation. Ventricular enlargement consistent with hydrocephalus was present in 94% (31/33). Ventricle volume correlated with region specific transependymal CSF flow (periventricular T2 signal), localized anterior to the lateral ventricles. Subependymal tumor cells were also present subjacent to the 4th ventricle in a post-mortem human specimen. Conclusions: This is the first study to report CSF pathway tumor dissemination an animal model of DIPG and is representative of CSF dissemination seen clinically. Understanding the CSF-tumor-ependymal microenvironment has significant implications for treatment of DIPG through targeting mechanisms of tumor spread within the CSF pathways.

HGG-12. HUMAN IPSC-DERIVED H3.3K27M NEUROSPHERES: A NOVEL MODEL FOR INVESTIGATING DIPG PATHOGENESIS AND DRUG RESPONSE

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Diffuse intrinsic pontine glioma (DIPG) is a subset of high-grade glioma that occurs predominantly in children and has no cure. Up to 80% of DIPG harbor a heterozygous point mutation that results in a lysine 27 to methionine substitution in histone variant H3.3 (H3.3K27M). Existing DIPG models have provided insight into the role of H3.3K27M but have limitations: genetically engineered murine models often rely on overexpression of the mutant histone to form tumors; patient-derived xenografts (PDX) are more genetically faithful but preclude examination of the effect of individual mutations on pathogenesis. To address these shortcomings and better recapitulate the genetics of human tumors, we designed a novel DIPG model based on human induced pluripotent stem cells (iPSC) edited via CRISPR to express heterozygous H3.3K27M. Edited iPSC were chemically differentiated into neural progenitor cells, which upon implantation into the brainstems of immunodeficient mice formed diffusely invasive tumors that were histologically consistent with high-grade glioma. Further, neurospheres cultured from primary tumors formed secondary tumors upon reimplantation with more diffuse invasion, suggesting *in vivo* evolution. To validate this model's relevance to DIPG transcriptionally, we performed RNA-sequencing on a cohort of primary and secondary tumor neurospheres (termed primary and secondary iDIPG) and compared them to published RNA-seq data from pediatric PDX and patient tumor samples. Hierarchical clustering and principal component analysis on differentially expressed genes ($P<0.05$) showed that H3.3K27M iDIPG cluster with H3.3K27M PDX and patient tumors. Further, ssGSEA showed that H3.3K27M iDIPG are enriched for astrocytic and mesenchymal signature genes, a defining feature of H3.3K27M DIPG. Finally, we found that primary H3.3K27M iDIPG neurospheres are sensitive to panobinostat, an HDAC inhibitor shown to be effective against H3.3K27M DIPG cells *in vitro*. Overall, these data suggest that H3.3K27M iDIPG are a promising tool for investigating DIPG biology and new therapeutic strategies.