High-grade glomas, including diffuse intrinsic pontine glioma (DIPG), are a lethal group of cancers whose progression is strongly regulated by neuronal activity [Venkat 2015]. One way in which gloma cells sense neuronal activity is via interaction with the extracellular domain of post-synaptic adhesion protein neurogli-3 (NLGN3), which is cleaved and released into the tumor microenvironment (TME) by the sheddase ADAM10. This interaction drives gloma growth, but the relevant binding partner of shed NLGN3 (sNLGN3) on gloma cells is currently unknown. Here, we report that sNLGN3 binds to chondroitin sulfate pro- teoglycan 4 (CSPG4), in turn inducing released intramembrane proteolysis (RIP) of CSPG4, and initiating a signaling cascade within DIPG cells to promote tumor growth. CSPG4 RIP involves activity-regulated extracellular shedding by ADAM10 and subsequent gamma-secretase cleavage of the extracellular domain in healthy oligodendroglial cancer cells (OPCs), putative cells of origin for several forms of high-grade glioma [Sakry 2014]. Incubation of high-grade gloma cells or healthy OPCs with recombinant sNLGN3 or human serum sufficiently raises ADAM10-mediated extracellular release of CSPG4 and subsequent gamma-secretase-mediated cleavage of the CSPG4 intracellular domain (ICD). Pre-treatment of gloma cells or OPCs with an ADAM10 inhibitor entirely blocks NLGN3-induced CSPG4 shedding. Inhibition of CSPG4 via CRISPR/Cas9 prevents the growth-promoting effects of NLGN3 application in vitro. We are now performing experiments to better discern how the activated ICD regulates signaling consequences downstream of NLGN3 binding. In addition, we are using a surface plasmon resonance to investigate whether the shed ectodomains of NLGN3 and CSPG4 remain in complex or only transiently interact. Altogether, our data form a critical missing link in understanding how gloma cells sense, translate and respond to neuronal activity in the TME and identify a new therapeutic target to disrupt neuron-gloma interactions.

Diffuse intrinsic pontine glioma remains a devastating condition with a dismal five-year survival rate of less than 5%. Few models of DIPG incorporate the study of H3.1K27M despite the fact that this high-grade glioma that occurs in the brainstem with a median survival of less than two years is the most common embryonal tumor in children. Interestingly, H3.1K27M DIPG show an increased incidence in females, confirming by H and E staining, between 60–110 days post injection. Additionally, confirmed through immunofluorescence staining, we can isolate a putative cells of origin for several forms of high-grade glioma {Sakry 2014} and identify a new therapeutic target to disrupt neuron-glioma interactions.
Abstracts

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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 neuropheres. 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 neuropheres and had profound cytotoxic activity against a panel of DIPG cultures. In a DIPG xenograft model it was superior to control, with 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≤0.01)). This suggests that the same tumor in cortical region may respond to systemic treatment with CBL0137, however, temsirolimus led to a significant improvement in survival in animals with DIPG in brainstem compared to control. However, temsirolimus had profound cytotoxic activity against DIPG neuropheres. To determine if tumor location impacts therapeutic outcome, we performed in vivo and in vitro experiments comparing the CSF pathways of DIPG treated with CBL0137 or control. 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 both models the two agents had profound synergistic activity against DIPG neuropheres in clonogenic assays and enhanced caspase activation and apoptosis. The FACT subunit SSRP1 was found to directly interact with H3.3K27M and to target the mutant histone to form tumors; patient-derived xenografts showed similar results. Histone H3.3 trimethylation and leading to tumor cell death. Transcriptional analysis and immunoblotting indicated that combination treatment activated signalling pathways controlled by Retinoblastoma (RB)/E2F1 and subsequent increased phosphorylation and enzymatic activity of eukaryotic transcription factor 2 (ETH2). 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 reduced tumor cell proliferation 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-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 histopathology analysis with H&E and beta IV tubulin. Results: Tumor dissemination within the CSF pathways (ventricles, leptomeninges) was present in 76% (23/33) of animals, with invasion of the choroid plexus, disruption of the ciliated ependymal and regional subarachnoid space. Ventricular enlargement consistent with hydrocephalus was present in 94% (31/33). Ventricular volume correlated with region specific transpendymal CSF flow (p<0.05) localized anterior to the lateral ventricles. Subarachnoid space present subependymally in a post-mortem human specimen. Conclusions: This is the first study to report CSF pathways 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 DRD 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 H3.3.K27M.Edited iPSCs were chemically differentiated into neural progenitor cells, which upon implantation into the brains 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 an in vivo evolution. To validate this model, we performed transcriptomic analysis on differentially expressed genes (P<0.05) showed that H3.3.K27M DIPG cluster with H3.3K27M PDX and patient tumors. Further, ssGSEA analysis on differentially expressed genes (P<0.05) showed that H3.3.K27M DIPG clusters with H3.3K27M PDX and patient tumors. Further, ssGSEA of H3.3.K27M DIPG and PDX tumors for investigating DIPG biology and new therapeutic strategies.

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 high-grade glioma with median survival of only 12 months from diagnosis. Current therapies are essentially palliative. The blood-brain barrier (BBB) is a main obstacle to delivering effective chemotherapeutics to 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, while histological analysis showed reduced permeability in brainstem compared to cortical region. Single-cell RNA sequencing experiments are currently underway to quantify specific differences in brainstem 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, temsirolimus, or 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≤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 may be a 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 cerebrovascularule may potentially lead to a novel strategy to treat DIPG as well as other brain tumors.