synapses, reduces glioma proliferation in patient-derived DIPG xenografts. This emerging understanding of brain cancer neurophysiology reveals new therapeutic targets and highlights commonly used drugs about which more study is required in this disease context.

HGG-05. NEURONAL ACTIVITY PROMOTES PEDIATRIC HIGH-GRADE GLIOMA GROWTH THROUGH A NLGN3-CSPG4 SIGNALING AXIS

Shawn Gillespie, Yoon Kim, Anna Geraghty, Pamelyn Woo, and Michelle Monje; Stanford University, Stanford, CA, USA

High-grade gliomas, including diffuse intrinsic pontine glioma (DIPG), are a lethal group of cancers whose progression is strongly regulated by neuronal activity {Venkatesh 2015}{Venkatesh 2017}{Venkatesh 2019}. One way in which glioma cells sense neuronal activity is via interaction with the ectodomain of post-synaptic adhesion protein neuroligin-3 (NLGN3), which is cleaved and released into the tumor microenvironment (TME) by the sheddase ADAM10. This interaction drives glioma growth, but the relevant binding partner of shed NLGN3 (sNLGN3) on glioma cells is currently unknown. Here, we report that sNLGN3 binds to chondroitin sulfate proteoglycan 4 (CSPG4), in turn inducing regulated intramembrane proteolysis (RIP) of CSPG4, and initiating a signaling cascade within DIPG cells to promote tumor growth. CSPG4 RIP involves activity-regulated ectodomain shedding by ADAM10 and subsequent gamma secretase-mediated release of the intracellular domain in healthy oligodendroglial precursor cells (OPCs), putative cells of origin for several forms of high-grade glioma {Sakry 2014}{Nayak 2018}. Incubation of high-grade glioma cells or healthy OPCs with recombinant NLGN3 is sufficient to augment ADAM10-mediated ectodomain release of CSPG4 and subsequent gamma secretase-mediated cleavage of the CSPG4 intracellular domain (ICD). Pre-treatment of glioma cells or OPCs with an ADAM10 inhibitor entirely blocks NLGN3-induced CSPG4 shedding. Acute depletion of CSPG4 via CRISPR gene editing renders glioma cells insensitive to the growth-promoting effects of NLGN3 application in vitro. We are now performing experiments to better discern how the CSPG4 ICD regulates signaling consequences downstream of sNLGN3 binding. In addition, we are using 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 glioma cells sense, translate and respond to neuronal activity in the TME and identify a new therapeutic target to disrupt neuron-glioma interactions.

HGG-06. EARLY GABAERGIC NEURONAL LINEAGE DEFINES DEPENDENCIES IN HISTONE H3 G34R/V GLIOMA

Ilon Liu[1], Lynn Bjerke[2], Gustavo Alencastro Veiga Cruzeiro[1], Rebecca F. Rogers[2], Yura Grabovska[2], Eshini Panditharatna[1], Alan Mackay[2], Tara Barron[3], McKenzie Shaw[1], Samantha E. Hoffman[1], Olivia A. Hack[1], Michael A. Quezada[3], Joshua Dempster[4], Sara Temelso[2], Bernhard Englinger[1], Valeria Molinari[2], Hafsa M. Mire[1], Li Jiang[1], Sibylle Madlener[5], Lisa Mayr[5], Christian Dorfer[5], Rene Geyeregger[6], Christopher Rota[7], Gerda Ricken[5], Sanda Alexandrescu[8], Emelie Braun[9], Miri Danan-Gotthold[9], Lijuan Hu[9], Kimberly Siletti[9], Erik Sundstroem[9], Rebecca Hodge[10], Ed Lein[10], Sameer Agnihotri[11], David D. Eisenstat[12], Fernando Carceller[2], Simon Stapleton[13], Cristina Bleil[14], Angela Mastronuzzi[15], Kristina A. Cole[16], Angela J. Waanders[17], Angel Montero Carcaboso[18], Maria Vinci[15], Darren Hargrave[19], Christine Haberler[5], Johannes Gojo[5], Irene Slavc[5], Sten Linnarsson[9], Michelle Monje[3], Chris Jones[2], and Mariella G. Filbin[1]; [1]Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA, [2]Institute of Cancer Research, London, UK, [3]Stanford University School of Medicine, Stanford, CA, USA, [4]Broad Institute, Cambridge, MA, USA, [5]Medical University of Vienna, Vienna, Austria, [6]CCRI, Vienna, Austria, [7]Harvard Medical School, Boston, MA, USA, [8]Boston Children's Hospital, Boston, MA, USA, [9]Karolinska Institute, Stockholm, Sweden, [10]Allen Institute, Seattle, WA, USA, [11]University of Pittsburgh, Pittsburgh, PA, USA, [12]University of Melbourne, Parkville, VIC, Australia, [13]St, George's Hospital, London, UK, [14]King's College Hospital, London, UK, [15]Ospedale Pediatrico Bambino Gesù, Rome, Italy, [16]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [17]Lurie Children's Hospital of Chicago, Chicago, IL, USA, [18]Hospital Sant Joan de Déu, Barcelona, Spain, [19]UCL Great Ormond Street Institute for Child Health, London, UK

High-grade gliomas harboring H3 G34R/V mutations exclusively occur in the cerebral hemispheres of adolescents and young adults, suggesting a distinct neurodevelopmental origin. Combining multimodal bulk and single-cell genomics with unbiased genome-scale CRISPR/Cas9 approaches, we here describe a GABAergic interneuron progenitor lineage as the most likely context from which these H3 G34R/V mutations drive gliomagenesis, conferring unique and tumor-selective gene targets essential for glioma cell survival, as validated genetically and pharmacologically. Phenotypically, we demonstrate that while H3 G34R/V glioma cells harbor the neurotransmitter GABA, they are developmentally stalled, and do not induce the neuronal hyperexcitability described in other glioma subtypes. These findings offer a striking counter-example to the prevailing view of glioma origins in glial precursor cells, resulting in distinct cellular, microenvironmental, and therapeutic consequences.

HGG-07. RADIATION INDUCED SENESCENCE IN DIFFUSE INTRINSIC PONTINE GLIOMA CELLS REVEALS SELECTIVE VULNERABILITY TO BCL-XL INHIBITION

Ashley Vardon[1], Romain Guiho[1], Diana Carvalho[2], Chris Jones[2], Darren Hargrave[3,1], and Juan-Pedro Martinez-Barbera[1]; [1]UCL Great Ormond Street Institute of Child Health, London, UK, [2]The Institute of Cancer Research, Sutton, UK, [3]Great Ormond Street Hospital NHS Trust, London, UK

Diffuse intrinsic pontine glioma remains a devastating condition with a dismal five year survival rate less than 5%. New approaches for treating this aggressive disease are critical to driving progress. Conventional radiotherapy remains the cornerstone of treatment, with no chemotherapeutic agent found to improve survival. However, radiotherapy is often delivered as a palliative treatment, and disease often recurs 3–6 months after. Radiation causes DNA damage and oxidative stress yielding a senescent state of replicative arrest in susceptible cells. However, increasing evidence demonstrates malignant cells can escape senescence leading to tumour recurrence. Targeted ablation of non-replicating senescent tumour cells following radiation could negate tumour recurrence. It remains unknown whether DIPG undergoes senescence following radiation, and furthermore, whether senolytics can be utilised to target senescent DIPG cells. We employed radiation to induce a senescent state in primary human DIPG cell lines. Senescence was confirmed using SA-β-gal staining, lack of EdU incorporation and qRT-PCR to characterise the SASP in three primary human DIPG cell lines. RNA-sequencing on DIPG cells following radiation revealed senescence and SASP signatures. Viable cells that survive radiation were then utilised to screen candidate senolytic drugs, only Bcl-XL inhibitors demonstrated reproducible senolytic activity in radiation treated DIPG cells. Conversely, Bcl-2 inhibitors failed to show any consistent senolytic activity. These results demonstrate future possibilities of targeting radiation induced senescence in DIPG, using novel senolytic therapies and highlight Bcl-XL dependency as a potential vulnerability of surviving DIPG cells following exposure to radiation.

HGG-08. CREATION OF AN IN VITRO AND IN VIVO MODEL SYSTEM FOR THE STUDY OF H3.1K27M DIPG

Joseph Lagas[1], Lihua Yang[1], Oren Becher[2], and Joshua Rubin[1]; [1]Washington University in St. Louis, St. Louis, MO, USA, [2]Northwestern University, Evanston, IL, USA

Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating pediatric high-grade glioma that occurs in the brainstem with a median survival of less than 1 year. A greater understanding of the early tumorigenic events is essential for the development of effective therapeutics. DIPG is characterized by founder mutations in histone H3, either H3.1K27M or H3.3K27M. These mutations cause global hypomethylation, resulting in aberrant gene expression. Little is known about how this mechanism contributes to tumorigenesis. Interestingly, H3.1K27M DIPG show an increased incidence in females, whereas H3.3K27M DIPG shows no sex difference. This illustrates that the tumorigenic potential of H3.1K27M may be different between the sexes. Few models of DIPG incorporate the study of H3.1K27M despite the fact that it represents a unique opportunity to obtain valuable information on the tumorigenesis of DIPG through the study of the sex difference. Thus, we have created an *in vitro* and *in vivo* model system for H3.1K27M DIPG utilizing the RCAS mouse model system. This system utilizes RCAS vectors and a RCAS-ntva transgenic mouse line to deliver specific mutations to nestin expressing cells in the brainstem, including oligodendrocyte progenitor cells (OPCs), the predicted cell of origin. Delivering H3.1K27M, ACVR1 R206H, and PDGFaa at postnatal day 7 produces DIPG-like tumors *in vivo*, confirmed by H and E staining, between 60–110 days post injection. Additionally, confirmed through immunofluorescence staining, we can isolate a pure population of OPCs via immunopanning and infect them with RCAS vectors *in vitro* to produce stable expression of H3.1K27M. Introduction of H3.1K27M alone into male and female OPC cultures provides an opportunity to compare the early tumorigenic effects of H3.1K27M between the sexes *in vitro*. These results demonstrate that we have created an *in vitro* and *in vivo* H3.1K27M DIPG model system for the study of sex differences and tumorigenesis in DIPG.

HGG-09. TARGETING FACILITATES CHROMATIN TRANSCRIPTION (FACT) AS A NOVEL STRATEGY THAT ENHANCES RESPONSE TO HISTONE DEACETYLASE (HDAC) INHIBITION IN DIPG

Anahid Ehteda[1], Sandy Simon[1], Laura Franshaw[1], Federico M. Giorgi[2], Jie Liu[1], Elisha Hayden[1], Jourdin Rouaen[1],