LGG-54. ASTHMA REDUCES GLIOMA FORMATION BY T CELL DECORIN-MEDIATED INHIBITION OF MICROGLIA

Jit Chatterjee, Shilpa Sanapala, Olivia Cobb, Alice Bewley, Elizabeth Cordell, Joel Garbow, Michael Holtzman, David Gutmann; Washington University, St. Louis, MO, USA

To elucidate the mechanisms underlying the reduced incidence of brain tumors in children with Neurofibromatosis type 1 (NF1) and asthma, we leverage optic pathway glioma (*Nf1*-OPG) mice, human and mouse RNAseq data, and two different experimental asthma models. Following ovalbumin or house dust mite asthma induction at 4-6 weeks of age (WOA), *Nf1*-OPG mouse optic nerve volumes and proliferation are decreased at 12 and 24 WOA, indicating no tumor development. This inhibition is accompanied by reduced expression of the microglia-produced optic glioma mitogen, Ccl5. Human and murine T cell transcriptome analyses reveal that inhibition of microglia Ccl5 production results from increased T cell expression of decorin, which blocks Ccl4-mediated microglia Ccl5 expression through reduced microglia NFκB signaling. Decorin or NFkB inhibitor treatment of *Nf1*-OPG mice at 4-6 WOA inhibits tumor formation at 12 WOA, thus establishing a potential mechanistic etiology for the attenuated glioma incidence observed in children with asthma.

LGG-55. AUTOPHAGY SENSITIZES CNS TUMORS TO TARGETED THERAPY BY LOWERING THEIR APOPTOTIC THRESHOLD

Michele Crespo, Shadi Zahedi, Andrew Morin, Darya Wodetzki, Cecilia Caino, Jean Mulcahy Levy; University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Autophagy inhibition improves the effectiveness and overcomes RAF pathway inhibition (RAFi) resistance across multiple CNS tumors and molecularly distinct resistance mechanisms. Mechanistic links between autophagy and apoptotic cell death may explain this ability to improve RAFi response and reverse resistance. RAFi sensitive (MAF 794, AM38) and resistant (MAF 794R, MAF 905-3, AM38R, B76) BRAFV600E CNS tumor cell lines were analyzed at baseline, following RAFi (vemurafenib), autophagy inhibition (chloroquine or shRNAs), and combination therapy. Growth assays and caspase activation were monitored by Incucyte Zoom. qRT-PCR evaluated key pro-apoptotic BH3-only members of the BCL-2 family. Broad BH-3 profiling was completed using the Letai JC-1 Plate-Based protocol. Western blot analysis assessed protein levels. Combination pharmacologic treatment caused alterations in key pro-apoptotic BH3-only proteins including an increase in BNIP3L and PUMA. Genetically inhibiting autophagy with shRNAs for ATG5 and ATG7 (proteins required for formation of the autophagosome) produced similar results with increases in both protein and mRNA levels of BNIP3L and PUMA following RAFi treatment. This suggested autophagy-mediated regulation of BH3 proteins functions to determine cellular apoptotic threshold. Caspase activation demonstrated increased effectiveness of combined RAFi and autophagy inhibition overcoming the apoptotic threshold compared to single drug treatment. BH3 profiling demonstrated a dependence on BCL-2 to inhibit apoptosis. BH3 mimetics competitively bind to pro-survival BCL-2 family members, blocking their protective effects and pushing tumor cells towards apoptosis. Autophagy inhibition can also improve treatment response by overcoming the apoptotic threshold in RAFi resistant cells and magnifying the apoptotic response in sensitive cells. BH3 profiling reveals CNS BRAFV600E are BCL-2 dependent cells, unprimed for apoptosis, which may be good candidates for additional treatment with BH3 mimetics such as venetoclax. This presents an attractive treatment for MAPK activated CNS tumors by enhancing apoptotic cell death by targeting the MAPK pathway, autophagy and BH3.

LGG-56. SURGICAL MANAGEMENT OF PRE-CHIASMATIC INTRAORBITAL OPTIC NERVE GLIOMAS IN CHILDREN AFTER LOSS OF VISUAL FUNCTION – RESECTION FROM BULBUS TO CHIASM

Julian Zipfel[1,2], Jonas Tellermann[1], Dorothea Besch[3], Eckart Bertelmann[4], Martin Ebinger[5], Pablo-Hernandez Driver[6], Ulrich-Wilhelm Thomale[7], Martin U. Schuhmann[8,2]; [1]Section of Pediatric Neurosurgery, Department of Neurosurgery, Universtiy Hospital of Tuebingen, Tuebingen, Baden Wurttemberg, Germany. [2]Centre of Neurofibromatosis, Centre of Rare Diseases, University Hospital of Tuebingen, Tuebingen, Baden Wurttemberg, Germany. [3]Section of Periocular and Orbital Surgery, Department of Ophthalmology, University Hospital of Tuebingen, Tuebingen, Baden Wurttemberg, Germany. [4]Department of Opthalmology, Charité Universitaetsmedizin Berlin, Berlin, Berlin, Germany. [5]Department of Paediatric Oncology, University Children's Hospital of Tuebingen, Tuebingen, Baden Wurttemberg, Germany. [6]Department of Pediatric Oncology and Hematology, Charité Universitaetsmedizin Berlin, Berlin, Berlin, Germany. [7]Pediatric Neurosurgery, Charité Universitaetsmedizin Berlin, Berlin, Berlin, Germany. [8]Section of Pediatric Neurosurgery, Department of Neurosurgery, University Hospital of Tuebingen, Tuebingen, Baden Wurttemberg, Germany

INTRODUCTION: Optic pathway gliomas (OPG) in children carry significant morbidity and therapeutic challenges. The subgroup of pre-chiasmatic gliomas manifest with exopthalmus are a subgroup where, after blindness has occurred, an intraorbital and intradural resection is a curative option. We present a two-center cohort using two different surgical approaches and describe indication, technique, and long term surgical outcome. METHODS: A retrospective analysis in both centers was performed to included patients < 18 years at diagnosis with a pre-chiasmatic intra-orbital glioma, in whom a resection from the bulb to the chiasm was performed. RESULTS: 11 patients were included. 4 had NF1. Mean age at surgery was 7.0 years. Interval between diagnosis and surgery was 1-74 (median 10) months. Two had prior chemotherapy, one radiation, one both, one prior intraorbital surgery. In all 5 progression occurred. Indications for surgery were exophthalmos, pain, tumor progression or a combination. 8 patients (Group A) underwent an extradural trans-orbital-roof approach to resect intra-orbital tumour including the optic canal part plus intradural pre-chiasmatic resection. In 3 patients (Group B) a combined supra-orbital mini-craniotomy plus orbital frame osteotomy was used for intraorbital tumour-resection, excluding the optic canal part, plus intradural pre-chiasmatic resection. GTR was achieved in 7/8 of Group A and none had a recurrence (mean-FU 42 month). One residual behind the bulbus showed progression, treated by chemotherapy. All residuals in Group B were remnants of the optic nerve within optic canal remained stable (mean FU 11.8 months). No patient had a chiasmatic functional affection or permanent oculomotor deficits. Two after prior radiotherapy developed slight enophthalmos. CONCLUSION: In these selected patients surgical resection from bulb to chiasm (± removal of optic canal tumor) is safe without long-term sequela and with excellent cosmetic result. Surgery removes immediately exophthalmos and provides an effective long-term tumor control. It should be considered therapy of choice.

LGG-58. UNDERSTANDING THE TRANSCRIPTIONAL HETEROGENEITY OF PEDIATRIC LOW-GRADE GLIOMAS AND ITS IMPLICATION FOR TUMOR PATHOPHYSIOLOGY

Michelle Boisvert[1,2], Ashwyn A. Perera[3,4], Alexandra L. Condurat[5,2], John Jeang[2,1], Jessica W. Tsai[6,7], Dana Novikov[6], Kevin Zhou[6], Madison Chacon[6], Jeromy DiGiacomo[6], Rushil Kumbhani[6], Dayle Wang[6], Michael D. Taylor[8], Jordan R. Hansford[9,10], Louise Ludlow[11,12], Nada Jabado[13,14], Keith L. Ligon[15,16], Rameen Beroukhim[15,2], Pratiti Bandopadhayay[6,2], David T.W. Jones[17,4]; [1]Department of Cancer Biology, Dana Farber Cancer Institute, Boston, MA, USA. [2]Broad Institute of MIT and Harvard, Cambridge, MA, USA. [3]German Cancer Research Center (DKFZ), Heidelberg, Germany. [4]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany. [5]Department of Pediatric Oncology, Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA. [6]Dana-Farber/Boston Children's Cancer and Blood Disorder Center, Boston, MA, USA. [7]Boston Combined Residency Program in Pediatrics, Boston, MA, USA. [8]Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Toronto, Canada. [9]Children's Cancer Centre, Royal Children's Hospital, Melbourne, Australia. [10]Department of Pediatrics, University of Melbourne, Melbourne, Australia. [11]Children's Cancer Centre, The Royal Children's Hospital, Melbourne, Australia. [12]Murdoch Children's Research Institute, The Royal Children's Hospital, Melbourne, Australia. [13]Department of Human Genetics, McGill University, Montreal, Canada. [14]Department of Pediatrics, McGill University, Montreal, Canada. [15]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. [16]Department of Pathology, Boston Children's Hospital and Brigham and Women's Hospital, Harvard Medical School, and Department of Oncologic Pathology, Dana-Farber Cancer Institute, Boston, MA, USA. [17]Clinical Cooperation Unit Neuropathology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

Pediatric low-grade gliomas (pLGGs) are the most frequent brain tumors in children and comprise a heterogeneous group of tumors with different locations, histologic subtypes, ages at presentation, and clinical behavior. Tumors frequently respond to treatment with chemotherapy or surgical removal, but they can regrow after a period of quiescence, requiring further therapy. Thus, a deeper understanding of the molecular processes involved in these tumors is required to develop therapeutic strategies that are effective against their disease mechanisms. To better understand the cellular behaviors of this heterogenous group of tumors, we have employed single-cell and single-nuclei RNA sequencing technologies to analyze a large-scale dataset (>250,000 cells) of pLGGs. Analysis of this data identified a heterogenous population of cell types and cell states, detecting mature and progenitor-like astrocytes and oligodendrocytes, as well as cells exhibiting senescence or cycling programs. Moreover, we identify a significant immune infiltrate, comprised primarily of microglia. In addition to heterogeneity within pLGG tumors, heterogeneity between LGG subtypes represents another layer that stratifies pLGG biology. We performed a compositional analysis of the cell types present in these tumors and compared transcription signatures and gene expression programs across shared cellular populations of histologically and genetically distinct pLGGs. Finally, we optimized our

integration and batch correction analyses by using external 293T cells as spike in controls during our single-cell and single-nuclei data generation steps to determine the most suitable method for batch-effect removal. Our analysis of human pLGGs at the single-cell and single-nuclei resolution provides critical insight into the heterogenous biological activities that constitute these tumors.

LGG-59. IDENTIFYING HIDDEN DRIVERS OF LOW-GRADE GLIOMA TUMOR GROWTH

Sean Misek[1,2], Aaron Fultineer[1], Alexandra Condurat[1,2], Kevin Zhou[1,2], David Root[1], Nicole Persky[1], Jesse Boehm[3,1], Pratiti Bandopadhayay[1,2], Rameen Beroukhim[1,2]; [1]Broad Institute, Cambridge, MA, USA. [2]Dana-Farber Cancer Institute, Boston, MA, USA. [3]Massachusetts Institute of Technology, Cambridge, MA, USA

Genomic drivers of pediatric low-grade gliomas (pLGGs) converge on alterations that activate the MAPK pathway. However, expression of individual driver oncogenes fails to induce tumor formation with high penetrance and, paradoxically, expression of these oncogenes suppresses growth in vitro. This, combined with the non-monotonic tumor growth rate in patients, suggests that there are "hidden drivers" beyond a single driver oncogene that are necessary to support tumor growth. The goal of this project is to leverage high-throughput functional genomics strategies to identify these hidden drivers of pLGG. Our preliminary data indicates that genes which modulate differentiation are required for the survival of LGG cells, suggesting that these genes may be hidden drivers of LGG tumor growth. Additionally, we hypothesize that secreted factors in the tumor microenvironment regulate pLGG tumor growth, potentially by modulating differentiation. In total, genes which cooperate with pLGG driver oncogenes to promote tumor growth may represent a new class of therapeutic targets and may explain the complex patterns of tumor growth that are observed in patients.

LGG-60. DEVELOPMENT AND IMPLEMENTATION OF A COMPLEMENTARY DIAGNOSTIC TOOL TO DETECT TARGETABLE PATHWAYS IN PEDIATRIC GLIOMA PATIENTS

Liana Nobre, Adrian Levine, Scott Milos, Monique Johnson, Benjamin Laxer, Scott Ryall, Robert Siddaway, Uri Tabori, Cynthia Hawkins; SickKids, Toronto, Ontario, Canada

Pediatric Low grade gliomas are mainly driven by MAPK alterations including mutations in BRAF (BRAF fusions and BRAFV600) and FGFR. This has led to the study of BRAF, MEK and more recently FGFR inhibitors resulting in variable responses. We hypothesize that differing levels of RAS_MAPK coupled with alternate pathway activation may be driving this variability. To address this, we designed a custom NanoString assay that integrates proteomic and transcriptomic profiling of 4 key cancer-related, actionable pathways (MAPK, PI3K-AKT-mTOR, JAK-STAT, and NFkB) with robust results on formalin-fixed paraffin embedded tissue, including archival samples up to approximately 15 years old. We validated this assay using 15 gold standard cell lines with defined changes in each pathway including both isogenic activating mutations and perturbation with inhibitors. These findings were confirmed using data from the Cancer Cell Line Encyclopedia. The panel was further validated using a cohort of 40 low grade glioma samples with available RNAseq data where the RNA expression signatures had high concordance between assays. We have currently run the assay on over 200 surgical tumor samples, including 206 gliomas, 15 ependymomas, 11 medulloblastomas, 14 high grade gliomas and 10 control normal brain specimens. Findings indicate significant variability in pathway activations between tumors, although PLGG overall have higher MAPK activation scores than control tissue and other tumor types, a subset of these tumors have increased activity in PIK , JAK and NFKB pathways, underscoring the importance of integrating transcriptomic and proteomic information in precision oncology treatments. Finally, single cell RNA sequencing data from pilocytic astrocytomas demonstrates significant heterogeneity in pathway activation states within the tumor cells, as well as high pathway activations in some tumor associated microglia. This raises further research questions regarding the role of tumor heterogeneity in treatment failures and the impact of targeted therapies on the tumor immune microenvironment.

LGG-61. CEREBROSPINAL FLUID AS A SOURCE FOR LIQUID BIOPSY IN PEDIATRIC GLIOMAS

Liana Nobre[1], Monique Johnson[1], Robert Siddaway[1], Javal Sheth[1], Scott Ryall[1], Michelle Ku[1], Rodney Lyn[1], Ana Stucklin[2], Michal Zapotocky[3], Uri Tabori[1], Cynthia Hawkins[1]; [1]SickKids, Toronto, Ontario, Canada. [2]Universitäts- Kinderspital Zürich, Zurich, Switzerland. [3]University Hospital Motol, Prague, Czech Republic

Central nervous system neoplasms are currently the leading cause of morbidity and mortality among childhood cancers, gliomas account for 50% of these cases. The last decade has seen a massive growth in our understanding of the genetic underpinnings of these cancers, including the discovery of multiple diagnostic, prognostic and therapeutic markers. However, molecular characterization of these tumours requires a biopsy, with no added therapeutic benefit particularly in unresectable tumors. Liquid biopsy is a minimally-invasive alternative to biopsies which enables molecular characterization to diagnose, monitor response to therapy, and potentially predict progression/recurrence. We here present the results of a customized capture based NGS panel including 21 commonly altered genes present in pediatric and AYA gliomas coupled with low pass whole genome as a diagnostic and monitoring liquid biopsy tool. To assess for common fusions, exonic and intronic regions of specific genes are covered to capture different breakpoints. To establish the sensitivity and specificity of this assay we have used a commercially available control (SeraseqR) with 18 known mutated genes of interest and a in house control sample with two additional mutations. Samples with low ctDNA concentration (10 ng) and a limit of detection as low as 0.5% variant allele frequency, had a sensitivity of 83% and specificity of 100%. At higher concentrations (30 ng of ctDNA) we achieved a sensitivity and specificity of 100 %. We are currently finalizing the validation steps ctDNA samples extracted from CSF collected intra-surgically, through ventricular shunt or lumbar puncture. Twenty-two samples have been tested with additional 40 samples in processing. Driver alterations were identified in 16/22 samples, with additional 3/4 samples having concordant CNV alterations between tumor and CSF. This work supports further implementation of CSF use as a minimal invasive source of diagnostic and monitoring sample in children and adolescent patients with gliomas.

LGG-62. WEIGHT CHANGE IN PEDIATRIC PATIENTS TREATED WITH MEK INHIBITORS: A RETROSPECTIVE COHORT STUDY

Hye Hyun Bahng[1], Jemily Malvar[2], Yueh-Yun Chi[2], Celia Framson[2], Nathan Robison[2,1]; [1]Keck School of Medicine of the University of Southern California, Los Angeles, CA, USA. [2]Children's Hospital Los Angeles, Los Angeles, CA, USA

BACKGROUND: MEK inhibition is an emerging treatment strategy in pediatric tumors characterized by activation of the Ras-Raf-MEK-ERK pathway, including low-grade glioma (LGG) and neurofibromatosis 1 (NF1)-associated tumors. Preliminary clinical experience suggests that MEK inhibitors (MEKi) may be associated with weight gain in children, which has not been a reported toxicity in adults. METHODS: 35 patients > 1 and < 21 years old treated at CHLA with MEKi between October 2013 and May 2019 were identified. Data was collected at t = 0 (baseline), t = 3 months, t = 6 months, t = 12 months, and t = 24 months, as well as pre- and post-treatment time points. Weight change was categorized as no change (change in Z-score [-0.25, +0.25]), weight gain (change in Z-score > 0.25), and weight loss (change in Z-score > -0.25). RESULTS: Weight gain and weight loss were seen in 11 (34.4%) and 8 (25%) patients, respectively, after 6 months on therapy. Weight gain reversed in 4 out of 5 patients with post-treatment data. There was no clear association between weight outcome and hypothesized covariates (including hypothalamic location and NF1 status). Notably, significant weight gain was seen across baseline weight spectrum, including patients who had underweight and severely overweight BMI percentiles at baseline. CONCLUSION: Our findings preliminarily suggest that MEK inhibition may be associated with clinically significant weight change, especially weight gain, in a subset of children and young adults. Reversal upon drug cessation suggests a causal relationship. Further prospective and mechanistic investigation is needed.

LGG-63 MEK INHIBITOR-ASSOCIATED RETINOPATHY (MEKAR) IN A PEDIATRIC PATIENT WITH AN OPTIC PATHWAY GLIOMA

Anne Bendel[1], Mary Skrypek[1], Heather Johnson[1], Jonathan Pribila[2]; [1]Children's Minnesota, Minneapolis, MN, USA. [2]Park Nicollet, St. Louis Park, MN, USA

Pediatric low-grade glioma (LGG) and plexiform neurofibroma (PN) universally have up-regulation of the RAS-mitogen-activated protein kinase (MAPK) pathway. Recent phase I and II clinical trials evaluating MEK inhibitors for the treatment pediatric LGG and PN report efficacy and tolerable side effects, including no reported ophthalmologic toxicity. Contrary to the pediatric experience, adult trials using MEK inhibitors describe sev-