Steve Hicks School of Social Work, Austin, TX, USA, [3]Sweet Tea Cancer Connections, Seattle, WA, USA, [4]Furman University, Greenville, SC, USA

Addressing family needs for social/emotional support is part of the duty of oncology care teams. This research presents a (2020) scoping review and a (2019) focus group initiated to explore pediatric neuro-oncology parent experience of social/emotional support in conjunction with developing an online peer application to address family needs. Currently, the value of online support is in the forefront of clinical conversation. The focus group queried eight parents whose children were under neuro-oncology treatment in the Northwest USA. Thematic findings include—parents want supportive peers who have (1) a personal and deep understanding of parenting a child with serious illness (they "get it"); (2) particular characteristics and skills that promote and sustain relationships, including—(a) good social skills, (b) ability to engage in "balanced" (cancer/non-cancer) conversations, (c) individual similarities (beliefs, age of children, cancer diagnosis/treatment), (d) logistic commonalities (location, availability), (e) pro-social personal characteristics (i.e. sense of humor, emotional/social flexibility), and an (f) ability to navigate and maintain social/emotional boundaries. Parents also initiated discussion about "the burden of supportive relationships" and supporting families doing "normal" activities without worrying about treatment side effects and contagions. The literature review supports finding (1) above; reveals the paucity of evidence-based supports available to this population; underscores the critical need for practitioners and researchers to develop more evidence-based supports and interventions for families of children experiencing cancer; and supports practitioners' consistently assessing parent and sibling social and emotional needs and then consistently referring or intervening when needs are identified.

TUMOR BIOLOGY (NOT FITTING A SPECIFIC DISEASE CATEGORY)

TBIO-01. SEX DIFFERENCES IN REDOX STATE UNDERLIE GLUTAMINE DEPENDENCY IN MALE GLIOBLASTOMA

Jasmin Sponagel[1], Shanshan Zhang[1], Prakash Chinnaiyan[2], Joshua Rubin[1], and Joseph Ippolito[1]; [1]Washington University School of Medicine, St. Louis, MO, USA, [2]Oakland University William Beaumont School of Medicine, Royal Oak, MI, USA

Glioblastoma (GBM) is an aggressive brain tumor in children and adults. It occurs more commonly in males, but female patients survive significantly longer. Understanding the molecular mechanisms that underlie those sex differences could support novel treatment strategies. In this regard, we found that male and female GBM patient samples differ in their metabolite abundance and that males exhibit a significantly higher abundance of amino acid metabolites. We confirmed those findings in a murine model of GBM, which has previously yielded important insights into sexual dimorphism in GBM. Furthermore, we found that male GBM cell cultures are significantly more sensitive to amino acid deprivation, which was almost entirely driven by amino acids involved in the synthesis of the antioxidant glutathione. Glutaminase 1 (GLS1) mediates the conversion from glutamine to glutamate, a crucial component of glutathione. We found that male GBM cells exhibited higher levels of GLS1, suggesting they are more dependent on glutamate. Indeed, we found that male GBM cells are more sensitive to pharmacological GLS1 inhibition with the clinical inhibitor CB-839. This correlated with significantly increased reactive oxygen species (ROS) in males compared to females. We further confirmed sex differences in redox state through pharmacological depletion of glutathione that resulted in a significant increase in ROS and cell death in male GBM. Together, these data indicate that male GBM cells are more dependent on glutamine to regulate ROS levels. This reveals novel sex-specific metabolic targets for GBM and underlines the importance of considering sex in metabolic targeting approaches.

TBIO-02. IMMUNE PROFILING OF RARE EMBRYONAL BRAIN TUMORS REVEAL EVIDENCE OF DYSREGULATED INTERFERON SIGNALLING AS A POTENTIAL DETERMINANT OF IMMUNOLOGICAL HETEROGENEITY

Dean Popovski[1], Fupan Yao[1], Andrew Bondoc[1], Salma Al-Karmi[1], Ben Ho[1], Holly Lindsay[2], Xiao-Nan Li[2,3], James Rutka[1,4], and Annie Huang[1,4]; [1]Arthur and Sonia Labatt Brain Tumour Centre, Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada, [2]Department of Pediatrics, Division of Hematology and Oncology, Baylor College of Medicine, Texas Children's Hospital, Houston, TX, USA, [3]Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA, [4]Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada

Embryonal brain tumors (EBTs) remain the most common malignant pediatric brain tumors. Despite recent advances and improved understanding of the molecular biology of EBTs, clinical outcomes remain poor for rare EBTs. Previous large-scale genomic studies of rare EBTs have shed light on distinct genomic, transcriptomic and epigenomic profiles. Interestingly, these studies have revealed prominent tumor heterogeneity that provides opportunity to develop novel treatment strategies to improve patient outcomes. To examine the tumor microenvironment and identify tumor- specific biological dependencies, we performed deconvolution analysis of bulk gene expression (171 RNA-seq, 236 microarrays) and 586 methylation arrays, which revealed significant intra and inter-tumoral heterogeneity and implicated interferon (IFN)-mediated signalling as a determinant of a distinct immunological profile in rare EBTs. To further elucidate the importance of IFN signalling, we performed scRNA-seq on 20 primary samples, which provided evidence of a spectrum of IFN-immunological responses that vary from immunosuppressive to immunologically exhaustive that occur in a host dependent manner. To further validate our findings, we utilised a genetically engineered murine model of Atypical Teratoid Rhabdoid Tumor and primary xenografts in humanised mice to corroborate our in-silico profiles in vivo. Through amalgamation of our in-silico data with our in vivo data, we have identified evidence that dysregulated IFN responses represent a core element of the immunological heterogeneity present within subsets of rare EBTs. An improved understanding of the immune milieu in rare EBTs will provide avenues to develop specific onco-immune targets to address this clinical need.

TBIO-03. THE GIFT FROM A CHILD PROGRAM IS EMPOWERING POST-MORTEM TISSUE DONATION ACROSS THE UNITED STATES

Angela Waanders[1,2], Melissa Williams[1], Nitin Wadwhani[1,2], Xiao-Nan Li[1], Stewart Goldman[1,2], Kella Tran-Du[3,2], Jane Minturn[3,2], Jennifer Mason[3,2], Mariarita Santi[3,2], Angela Viaene[3,2], Mateusz Koptyra[3,2], Michelle Monje-Deisseroth[4,2], Javad Nazarian[5,2], Madhuri Kambhampati[5], Augustine Eze[5], Mark Souweidane[6], Nicole Lyons[7], Ginny McLean[7], Patti Gustafson[7], and Allen Gustafson[7]; [1]Ann & Robert H. Lurie Children's Hospital, Chicago, IL, USA, [2]Children's Brain Tumor Network (CBTN,org), Philadelphia, PA, USA, [3]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [4]Stanford University/Lucile Packard Children's Hospital, Palo Alto, CA, USA, [5]Children's National Health System, Washington DC, USA, [6]Pediatric Brain and Spine Center, Weill Cornell Medicine, New York, NY, USA, [7]On behalf of Swifty Foundation and Gift from a Child (giftfromachild.org), Chicago, IL, USA

The Gift from a Child (GFAC) program was inspired by the dream of one child to donate his brain for research, recognizing the need to study tumor tissue collected at diagnosis, recurrence, and at the time of death. Founded by the Swifty Foundation in 2016, GFAC currently is comprised of five "Centers of Excellence" at institutions with expertise in pediatric neuro-oncology. Partnering with the Children's Brain Tumor Network, the program's mandate is twofold: make it possible for families to donate no matter where they live in the United States and make tissue available to scientists globally to empower discovery. In order to overcome barriers that have stifled postmortem collection in the past, GFAC has invested in Tissue Navigators - individuals at each center who coordinate all aspects of donation and communicate with families, medical providers, and laboratory scientists. In 2019 alone, GFAC coordinated 55 autopsy collections from multiple diagnosis. A key metric of the program is also capturing the global sharing and usage of each tissue sample, ensuring that tissue isn't simply "banked" but is actively being actively used to help unravel tumor biology. To date, tissue has been used for genomic and molecular data generation, preclinical model development including cell lines and PDX models, and for novel drug screening. Together with Children's Brain Tumor Network, the Gift from a Child program is helping to ensure the most precious gift that a family can make is used to accelerate the path to cures.

TBIO-05. GENOME-SCALE NUCLEOTIDE-SPECIFIC CHARACTERIZATION OF 5-HYDROXYMETHYLCYTOSINE IN PEDIATRIC CENTRAL NERVOUS SYSTEM TUMORS

Nasim Azizgolshani[1], Curtis L. Petersen[1], Lucas Salas[1], Youdinghuan Chen[1], Laurent Perreard[1], Lananh N. Nguyen[2,3], and Brock C. Christensen[1]; [1]Dartmouth College, Hanover, NH, USA, [2]Dartmouth Hitchcock Medical Center, Lebanon, NH, USA, [3]University Health Network, Toronto General Hospital, Toronto, Ontario, Canada

Though aberrant cytosine modifications are prevalent in cancer, nucleotide-specific 5-hydroxymethylcytosine (5hmC) modifications remain understudied, including in pediatric CNS tumors. Brain 5-hydroxymethylation is linked with development and differentiation. We measured genome-scale nucleotide-specific 5hmC in patients with diagnoses of glioma, ependymoma, and embryonal tumors under age 18 (n=36), and in non-tumor pediatric brain tissues (n=3). DNA was processed with tandem oxidative (OxBS) and bisulfite (BS) treatments followed by hybridization to the Illumina Methylation EPIC Array that interrogates over 860,000 CpG sites. We used the OxyBS R package to determine levels of 5hmC and 5mC. Mean 5hmC levels were lower in tumors (gliomas 4.1%, ependymomas 3.9%, and embryonal

tumors 3.4%) compared to nontumor tissues (5.3%). We subset to the CpGs with the 5% highest 5hmC content for downstream analyses (37,173 CpGs). These sites were enriched among regulatory elements, including TFBS (Odds Ratio 1.14 p-value 3.57E-20) and super-enhancers (OR 1.93, p-value 1.14E-126). Linear mixed-effects models adjusted for age, sex, and cell type proportions tested the CpG-specific differences in 5hmC between tumor and nontumor samples, as well as between tumor subtypes. 5hmC levels were depleted across tumors compared with nontumor brain tissues, including at CpG islands. Model-based clustering (RPMM) results indicated that patients with low 5hmC patterns have poorer overall survival and increased risk of recurrence. Our results indicate that 5hmC localizes to sites in the DNA critical to gene regulation and is associated with patient outcomes. This study offers an opportunity to potentially contribute to classification markers for childhood brain tumors.

TBIO-06. BDNF-TRKB SIGNALING REGULATES NEURON-GLIOMA SYNAPTOGENESIS AND PROMOTES TUMOR PROGRESSION

Kathryn Taylor, Helena Zhang, Alexa Hui, Shawn Gillespie, and Michelle Monje; Stanford, Stanford, CA, USA

Pediatric high-grade gliomas (pHGG) are a devastating group of diseases that urgently require novel therapeutic options. We have previously demonstrated that pHGGs hijack mechanisms of brain development and plasticity to their advantage. Here, we investigated the role of microenvironmental BDNF on pediatric gliomas, independent of the NTRK fusion events commonly identified in infant HGG. Genetic deletion or pharmacological blockade of *NTRK2* (TrkB), in patient-derived pediatric glioma increases survival in multiple DIPG and pGBM patient-derived orthotopic xenograft models. Unlike the paracrine BDNF-TrkB signaling observed between subpopulations of adult HGG malignant cells, pediatric glioma express TrkB, but not BDNF ligand. BDNF is secreted by normal brain cells in response to neuronal activity and conditioned medium experiments from cortical slices of mice indicates the brain microenvironment as the chief source of BDNF ligand. Addition of recombinant BDNF protein increases pediatric glioma cell proliferation and activates the canonical downstream MAPK signaling pathway, an effect that is blocked by genetic or pharmacological TrkB inhibition in pHGG. However, the glioma growth-promoting effects of BDNF in vivo cannot be explained by stimulation of MAPK signaling alone. We therefore examined the effects of BDNF signaling on neuron-to-glioma synapse formation, a newly recognized microenvironmental interaction important for pediatric glioma progression. We find that BDNF-TrkB signaling promotes neuron-to-glioma synaptogenesis in neuron-glioma co-culture. We are presently exploring the role for BDNF-TrkB signaling in glioma synaptic plasticity and function. Funding: Abbie's Army Foundation

TBIO-07. SINGLE-CELL TRANSCRIPTOMIC PROFILE REVEALS MACROPHAGE HETEROGENEITY IN SONIC-HEDGEHOG MEDULLOBLASTOMA AND THEIR DISTINCT RESPONSES TO DIFFERENT TREATMENT MODALITIES

Mai Dang[1], and Malay Haldar[2]; [1]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA

Tumor-associated macrophages (TAMs) are an important component of the tumor microenvironment. Pro-inflammatory macrophages can suppress while anti-inflammatory macrophages can promote tumor growth. Despite their abundance in many tumors, the origins and diversity of TAMs are not well understood, especially in pediatric brain tumors. Using single-cell RNA sequencing in a genetically engineered mouse model (Ptch+/-:p53-/-) of SHH-MB, we identified the dual microglia and monocytic origin of macrophage and their transcriptomic heterogeneity. We demonstrate differential recruitment and function of macrophages under distinct modalities of tumor therapy of molecular targeted hedgehog inhibition versus radiation. We additionally identify a monocytic macrophage population recruited post-radiation that is immune suppressive, suggesting a mechanism for radiation treatment failure. These insights uncover potential strategies for immunomodulation as adjunctive therapy for radiation.

TBIO-08. BASE-RESOLUTION METHYLOMES OF GLIOMAS BEARING HISTONE H3.3 MUTATIONS REVEAL A G34 MUTANT-SPECIFIC SIGNATURE SHARED WITH BONE TUMORS

Yuhei Sangatsuda[1], Fumihito Miura[2], Hiromitsu Araki[2], Masahiro Mizuguchi[1], Nobuhiro Hata[1], Daisuke Kuga[1], Ryusuke Hatae[1], Yojiro Akagi[1], Takeo Amemiya[1], Yutaka Fujioka[1], Yasuhito Arai[3], Tatsuhiro Shibata[3], Koji Yoshimoto[4], Takashi Ito[2], and Koji Iihara[1]; [1]Department of Neurosurgery, Kyushu University, Fukuoka, Japan, [2]Department of Biochemistry, Kyushu University, Fukuoka, Japan, [3]Division of Cancer Genomics, National Cancer Center Research Institute, Tokyo, Japan, [4]Department of Neurosurgery, Kagoshima University, Kagoshima, Japan

BACKGROUND: Two recurrent mutations, K27M and G34R/V, in H3F3A, encoding non-canonical histone H3.3, are reported in pediatric and young adult gliomas, whereas G34W mutation was prevalent in bone tumors. In contrast to K27 mutation, it remains elusive how G34 mutations affect the epigenome. Here we performed whole-genome bisulfite sequencing of four G34R-mutated gliomas and the G34V-mutated glioma cell line KNS-42. Similarly, we analyzed seven and three gliomas harboring K27M and no mutations in H3F3A, respectively. These data were compared with those on bone tumors. RESULTS: G34R-mutated gliomas exhibited lower global methylation levels, similar CpG island (CGI) methylation levels, and compromised hypermethylation of telomere-proximal CGIs compared with those bearing K27M and no mutations. Hypermethylated regions specific to G34R-mutated gliomas were enriched for CGIs, including those of OLIG1, OLIG2, and canonical histone genes in the HIST1 cluster. These CGIs were hypermethylated in osteosarcomas with, but not without, the G34W mutation. In KNS-42 cells, CGIs with G34V-mutated histone H3.3 exhibited higher methylation levels than those with wild-type histone H3.3. This effect was also observed in the G34R-mutated glioma samples. CONCLUSIONS: Gliomas bearing G34R/V mutations display characteristic methylomic alterations, some of which are shared by osteosarcomas with the G34W mutation. Deposition of G34 variants may lead to elevated methylation of otherwise hypomethylated, histone H3.3-bearing CGIs.

TBIO-09. *IN SILICO* ANALYSIS IDENTIFIES A PUTATIVE CELL-OF-ORIGIN FOR *BRAF* FUSION-POSITIVE CEREBELLAR PILOCYTIC ASTROCYTOMA

Subhi Talal Younes; University of Mississippi Medical Center, Jackson, MS, USA

Childhood cancers are increasingly recognized as disorders of cellular development. This study sought to identify the cellular and developmental origins of cerebellar pilocytic astrocytoma, the most common brain tumor of childhood. By leveraging publicly available gene expression data from such tumors and controlling for driver mutations, a set of eight known neuro-developmental genes were identified as being upregulated in cerebellar pilocytic astrocytoma. Mapping those genes onto mouse neurodevelopmental atlases identified significant overlap in their expression within the ventricular zone of the cerebellar anlage. Further analysis with a single cell RNA-sequencing atlas of the developing mouse cerebellum defined this overlap as occurring in ventricular zone progenitor cells at the division point between GABA-ergic neuronal and glial lineages, a developmental trajectory which closely mirrors that previously described to occur within pilocytic astrocytoma cells. Furthermore, ventricular zone progenitor cells and their progeny exhibited evidence of MAPK pathway activation, the paradigmatic oncogenic cascade known to be active in cerebellar pilocytic astrocytoma. Gene expression from developing human brain atlases recapitulated the same anatomic localizations and developmental trajectories as those found in mice. Taken together, these data suggest this population of ventricular zone progenitor cells as the cell-of-origin for *BRAF* fusion-positive cerebellar pilocytic astrocytoma.

TBIO-11. DEEP LEARNING-BASED SINGLE-CELL RNA SEQUENCING DIFFERENTIATION IDENTIFIES SIMPLE AND COMPLEX TRANSCRIPTIONAL NETWORKS FOR SUBPOPULATION CLASSIFICATION

Eric Prince, and Todd Hankinson; Children's Hospital Colorado, Aurora, CO, USA

BACKGROUND: Genomic assays capable of cellular resolution (i.e. scRNA-seq) are becoming ubiquitous in biomedical research. Machine learning, and the subtype known as Deep Learning, have broad application within scRNA-seq analytics. However, methods to facilitate the classification of cell populations are lacking. We present the novel computational framework HD Spot, which generates interpretable and robust Deep Learning classifiers that enable unbiased interrogation of linear and non-linear genomic signatures. METHODS: HD Spot is written in python and relies on Google's TensorFlow2 deep learning framework. Four datasets of immune cells were obtained from the publicly available Seurat repository, generated using the 10X chromium platform. Data preprocessing used standard Seurat methodology. HD Spot generated optimized classifiers via a custom platform. Network interpretability was achieved using Shapley values. Ontology analysis was performed using Metascape. RESULTS: HD Spot identified meaningful ontologic signatures across all tested datasets. In the binary case of control versus IFN-B stimulated CD4+ T cells, gene ontologies reflected T_{h0} and T_{h2} T cell populations, congruent with T cell activation. In the 9-class case of PBMCs, HD Spot identified meaningful gene networks characteristic of the ground-truth populations using raw feature counts alone. When feature counts are processed into expression values, HD Spot demonstrates increased specificity of top genes and respective ontologies between subpopulations. CONCLUSION: This work introduces a broadly applicable computational tool for the advanced bioinformatician to decipher complex cellular heterogeneity (e.g., tumors) in an unbiased way. Additionally, HD Spot lowers the barrier for novice bioinformaticists to derive actionable insights from their data.