OMIC-09. MAPPING THE HISTONE MUTATIONAL LANDSCAPE ACROSS ADULT AND PEDIATRIC CANCER GENOMES UNCOVERS NOVEL SOMATIC MUTATIONS IN PEDIATRIC HIGH-GRADE GLIOMAS

Erin Bonner[1,2], Krutika Gaonkar[3], Payal Jain[3], Yuankun Zhu[3], Sandra Laternser[4], Augustine Eze[1], Madhuri Kambhampati[1], Sridevi Yadavilli[1], Jason Cain[5], Sabine Mueller[4,6], Sebastian Waszak[7,8], and Javad Nazarian[4,1]; [1]Children's National Medical Center, Center for Genetic Medicine Research, Washington, DC, USA, [2]The George Washington University, Institute for Biomedical Sciences, Washington, DC, USA, [3]Children's Hospital of Philadelphia, Center for Data Driven Discovery in Biomedicine, Philadelphia, PA, USA, [4]University Children's Hospital Zürich, Department of Oncology, Zurich, Switzerland, [5]Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, Australia, [6]University of San Francisco California, Department of Neurology, Neurosurgery and Pediatrics, San Francisco, CA, USA, [7]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo and Oslo University Hospital, Oslo, Norway, [8]Department of Pediatric Research, Division of Paediatric and Adolescent Medicine, Rikshospitalet, Oslo University Hospital, Oslo, Norway

There is a growing role for mutations affecting histone linker and histone core-encoding genes across several adult and pediatric cancers. However, the extent to which somatic histone mutations may bridge across different cancers as common tumorigenic events – particularly in the context of pediatric CNS tumors – remains unclear. To address this knowledge gap, we set out to define a comprehensive pan-cancer landscape of somatic histone mutations. We first queried the ICGC PCAWG and TCGA Pan-Cancer Atlas representing >12,500 adult and pediatric cancer patients. We found lymphomas to be most enriched for histone mutations (50–75%) and, in particular, for mutations in linker histones (*HIST1H1B-E*), yet also in specific core histone genes (eg, *HIST2H2BE*). Moreover, we observed a significant enrichment of histone mutations in adult high-grade vs low-grade gliomas (10% *vs* 6%, $P<0.05$, n=922 patients). Interrogation of whole genome data from 800 pediatric CNS tumor genomes (PBTA/OpenDIPG), identified novel (non-H3K27/non-H3G34) somatic histone mutations in 5–10% of subjects, including pediatric high-grade gliomas (pHGGs) and diffuse midline gliomas (DMGs). We found an overlapping set of histone genes to be recurrently mutated in non-CNS cancers and pediatric CNS tumors alike (eg, *HIST1H1B/C/E*). Notably, the only pediatric primary CNS lymphoma patient also harbored a histone linker alteration (*HIST1H1B*), similar to adult non-CNS lymphoma patients. We validated novel somatic histone mutations in DMGs by Sanger sequencing. Ongoing studies include in vitro assessment of the impact of these mutations on cell proliferation, chromatin accessibility, histone spacing, and gene expression. In addition, we will further assess associations with clinical outcome, age, and tumor subtypes. Collectively, oncohistone vulnerabilities were identified and defined as histone gene families recurrently mutated across all cancer types. Our analyses of adult and pediatric cancer genomes have uncovered previously unknown mutations affecting histone linker and core proteins, which may play a yet-undefined role in tumor etiology.

OMIC-10. TRANSCRIPTOMIC ANALYSIS REVEALS SEX DIFFERENCES IN PEDIATRIC BRAIN MECHANISMS

Wei Yang[1], Justin D Lathia[2], James R Connor[3], Michael E Berens[4], Jill S Barnholtz-Sloan[5], and Joshua B Rubin[6,7]; [1]Department of Genetics, Washington University School of Medicine University School of Medicine, St Louis, MO, USA, [2]Rose Ella Burkhardt Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, Cleveland, OH, USA, [3]Department of Neurosurgery, Penn State College of Medicine, Hershey, PA, USA, [4]Cancer and Cell Biology Division, TGen, Phoenix, Arizona, USA, [5]Department of Population and Quantitative Health Sciences, Case Western Reserve University School of Medicine & University Hospitals, Cleveland, OH, USA, [6]Department of Pediatrics, Washington University School of Medicine, St Louis, MO, USA, [7]Department of Neuroscience, Washington University School of Medicine, St Louis, MO, USA

A significant male overrepresentation exists in cancer incidence and in cancer-related deaths. This is true in all regions of the world and across the lifespan. We published an analysis of adult glioblastoma transcriptomes in which we identified sex-biased molecular features that distinguished the longest surviving male and female patients. Male GBM was characterized by decreased expression of positive regulators of the cell, while female GBM was characterized by decreased expression of intermediates in integrin signaling. To determine whether similar sex differences exist in pediatric brain tumors (pBTs), we accessed 860 pBT transcriptomes, representing all diagnostic categories and ages through the Children's Brain Tumor Network. Unsupervised Bayesian nearest neighbor analysis of gene expression revealed distinct male and female expression patterns indicating fundamental differences exist in pBTs as a function of sex. Similar to our adult GBM analysis, male pBTs were distinguished from female pBTs by the involvement of cell cycle regulatory pathways. In contrast to adult GBM, female pBTs were characterized by involvement of metabolism and inflammatory/ immunity pathways. Interestingly, these sex differences were also evident in a parallel analysis of 209 of neuroblastoma cases. Focused analysis of the most common malignant pBTs (high-grade glioma, medulloblastoma, and ependymoma) revealed that each disease type exhibited significant sex differences in molecular profile, involving distinct pathways in each tumor type. Together, these data indicate that sex-based differences in molecular mechanisms exist in pBTs, and imply that sex-specific approaches to pBT treatment might yield improved outcomes for all patients.

OMIC-11. SINGLE CELL RNA SEQUENCING FROM THE CSF OF SUBJECTS WITH H3K27M+ DIPG/DMG TREATED WITH GD2 CAR T-CELLULAR THERAPY

Aaron Mochizuki, Sneha Ramakrishna, Zina Good, Shabnum Patel, Harshini Chinnasamy, Kristen Yeom, Liora Schultz, Rebecca Richards, Cynthia Campen, Agnes Reschke, Jasia Mahdi, Angus Toland, Christina Baggot, Sharon Mavroukakis, Emily Egeler, Jennifer Moon, Kayla Landrum, Courtney Erickson, Lindsey Rasmussen, Valentin Barsan, John Tamaresis, Anne Marcy, Michael Kunicki, Michelle Celones, Zach Ehlinger, Sreevidya Kurra, Timothy Cornell, Sonia Partap, Paul Fisher, Gerald Grant, Hannes Vogel, Kara Davis, Steven Feldman, Bita Sahaf, Robbie Majzner, Crystal Mackall, and Michelle Monje; Stanford University, Palo Alto, CA, USA

Introduction: We are conducting a Phase I clinical trial utilizing chimeric antigen receptor (CAR) T-cells targeting GD2 (NCT04196413) for H3K27M-mutant diffuse intrinsic pontine glioma (DIPG) and spinal cord diffuse midline glioma (DMG). Cerebrospinal fluid (CSF) is collected for correlative studies at the time of routine intracranial pressure monitoring via Ommaya catheter. Here we present single cell RNA-sequencing results from the first 3 subjects. Methods: Single cell RNA-sequencing was performed utilizing 10X Genomics on cells isolated from CSF at various time points before and after CAR T-cell administration and on the CAR T-cell product. Output was aligned with Cell Ranger and analyzed in R. Results: As detailed in the Majzner et al. abstract presented at this meeting, three of four subjects treated at dose-level one exhibited clear radiographic and/or clinical benefit. We have to date completed single cell RNA-sequencing for three of these four subjects (two with benefit, one without). After filtering out low-quality signals and doublets, 89,604 cells across 3 subjects were analyzed. Of these, 4,122 cells represent cells isolated from CSF and 85,482 cells represent CAR T-cell product. Two subjects who demonstrated clear clinical and radiographic improvement exhibited fewer $S100A8^+S100A9^+$ myeloid suppressor-cells and $CD25^+FOXP3^+$ regulatory T-cells in the CSF pre-infusion compared to the subject who did not derive a therapeutic response. In one subject with DIPG who demonstrated improvement, polyclonal CAR T-cells detectable in CSF at Day +14 demonstrated enrichment of CD8A, GZMA, GNLY and PDCD1 compared to the pre-infusion CAR T-cells by trajectory analysis, suggesting differentiation toward a cytotoxic phenotype; the same subject exhibited increasing numbers of $S100A8^+S100A9^+$ myeloid cells and $CX3CR1^+P2RY12^+$ microglia over time. Further analyses will be presented as data become available. Conclusions: The presence of immunosuppressive myeloid populations, detectable in CSF, may correlate to clinical response in CAR T cell therapy for DIPG/DMG.

OMIC-12. PREVALENCE AND SPECTRUM OF GERMLINE PATHOGENIC VARIANTS IN CANCER PREDISPOSITION GENES ACROSS THE CHILDREN'S BRAIN TUMOR NETWORK (CBTN)

Zalman Vaksman[1], Shelly McQuaid[2], Miriam Bornhorst[3], Yuankun Zhu[1], Allison Heath[1], Angela Waanders[2], Kristina Cole[1], Suzanne MacFarland[1], and Sharon Diskin[1]; [1]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [2]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA, [3]Children's National Hospital, Washington DC, USA

Germline variants are known to contribute to the pathogenesis of specific central nervous system (CNS) tumor subtypes; however, a large pan-pediatric brain and nervous system cancer germline susceptibility study has not been performed. To define the prevalence and spectrum of pathogenic variants in known cancer predisposition genes (CPGs; n=200), we analyzed whole genome sequencing (WGS) data from 880 pediatric subjects across 19 different cancer types in the Children's Brain Tumor Network (CTBN). Data were aligned using BWA. Variants were called using GATK and annotated with SnpEff and ANNOVAR. After quality control, variants with a minor allele frequency (MAF) < 0.1% in Gnomad 2.11 or ExAC were retained. Pathogenicity was assessed with American College of Medical Genetics (ACMG) guidelines using a lab-developed modification of ClinVar and InterVar. Automated pathogenic/likely pathogenic (P-LP) calls were manually reviewed by two cancer predisposition clinicians and a bioinformatician. Frequency of P-LP variants was assessed and gene burden testing was per-

formed against Gnomad3.1 (without cancer samples) using Fisher's exact test with Bonferroni adjustment. We observed 214 P-LP variants involving 190 unique individuals (21.6% of cohort). As expected, the most frequent variants were observed in *NF1, NF2,* and *TP53*(n=40 variants in 21% of individuals). *ATM*, *TSC2* and *CHEK2* variants (n=23) were observed in another 12% of individuals. An increased burden of P-LP variants was observed for 5 of these 6 genes ($p = 1.7x10^{-25}$ to $1.4x10^{-2}$, *CHEK2* $p=5.5x10^{-2}$). We also identified 5 variants in *BRCA2* (3 in high-grade glioma), 7 in REQC helicases (*BLM*, *WRN*, *REQL4*), and 16 variants in Fanconi anemia genes. Overall, cases harbored increased burden in P-LP variants in CPG genes ($p=8.8x^{-18}$) and the subset of DNA repair genes ($p=4.7x10^{-4}$). In conclusion we confirmed the association of variants in established predisposition genes while potentially identifying novel variants and genes associated in CNS tumors.

OMIC-13. THE ROLE OF COPY NUMBER ALTERATIONS IN PREDICTING SURVIVAL AND INFLUENCING TREATMENT OF CHILDHOOD BRAIN TUMORS

Sharon Freshour, Bryan Fisk, Christopher Miller, Obi Griffith, Malachi Griffith, and Joshua Rubin; Washington University School of Medicine, Saint Louis, MO, USA

Brain and central nervous system tumors are the most common form of solid tumor cancers and the second most common cancer overall among children. While many advances have been made in understanding the genomics of childhood brain tumors in recent years, the role of copy number alterations (CNAs) has not been fully characterized. Although the genomes of childhood brain tumor patients are generally considered to be relatively stable diploid genomes, analysis of a subset of pretreatment diagnostic samples from a cohort of 84 deceased patients from Washington University revealed widespread alterations, suggesting CNAs may play a larger role in the progression and prognosis of childhood brain tumors than originally thought. Follow up analysis of the entire cohort, containing a variety of tumor types that had low-pass whole genome sequencing performed, similarly showed evidence of CNAs across samples. 75 out 84 patients showed the presence of CNAs with an average of 16% of the genome being altered per sample and a median of 7%. Preliminary results examining correlations between the percentage of the genome that was copy number altered and event free survival or overall survival indicated that CNA percentage may have some prognostic value. For example, ependymoma samples showed positive correlation between alteration percentage and overall survival, while glioblastoma samples showed negative correlation. To explore copy number alteration in a larger cohort and increase statistical power, similar analyses are being performed using an additional 950 samples from the Pediatric Brain Tumor Atlas curated by The Children's Brain Tumor Network (CBTN) to determine if CNVs and CNV percentage or specific alterations can serve as prognostic markers and whether the biology of this genomic instability could inform therapeutic strategy.

OMIC-14. OPENPBTA: AN OPEN PEDIATRIC BRAIN TUMOR ATLAS

Joshua Shapiro[1], Candace Savonen[1], Chante Bethell[1], Krutika Gaonkar[2], Yuankun Zhu[2], Miguel Brown[2], Nhat Duong[2], Komal Rathi[2], Nighat Noureen[3], Bo Zhang[2], Brian Ennis[2], Stephanie Spielman[4], Bailey Farrow[2], Nicolas Van Kuren[2], Tejaswi Koganti[2], Shrivats Kannan[2], Pichai Raman[2], Daniel Miller[2], Payal Jain[2], Yiran Guo[2], Xiaoyan Huang[2], Adam Kraya[2], Allison Heath[2], Mateusz Koptyra[2], Jessica Wong[2], Jennifer Mason[2], Shannon Robbins[2], Mariarita Santi[2,5], Angela Viaene[2,5], Angela Waanders[6,7], Derek Hanson[8,9], Laura Scolaro[2], Hongbo Xie[2], Siyuan Zheng[3], Cassie Kline[2], Jena Lilly[2], Philip Storm[2], Adam Resnick[2], Jo Lynne Rokita[2], Casey Greene[1,10], and Jaclyn Taroni[1]; [1]Alex's Lemonade Stand Foundation, Philadelphia, PA, USA, [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [3]UT Health San Antonio, San Antonio, TX, USA, [4]Rowan University, Glassboro, NJ, USA, [5]University of Pennsylvania, Philadelphia, PA, USA, [6]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA, [7]Northwestern University Feinberg School of Medicine, Chicago, IL, USA, [8]Hackensack Meridian School of Medicine, Nutley, NJ, USA, [9]Hackensack University Medical Center, Hackensack, NJ, USA, [10]University of Colorado School of Medicine, Aurora, CO, USA

Pediatric brain tumors comprise a heterogeneous molecular and histological landscape that challenges most current precision-medicine approaches. While recent large-scale efforts to molecularly characterize distinct histological entities have dramatically advanced the field's capacity to classify and further define molecular subtypes, developing therapeutic and less toxic molecularly-defined clinical approaches remains a challenge. To define new approaches to meet these challenges and advance scalable, shared biospecimen- and data-resources for pediatric brain tumors, the Children's Brain Tumor Network and Pacific Pediatric Neuro-Oncology Consortium, in partnership with the Alex's Lemonade Stand Foundation Childhood Cancer Data Lab, launched OpenPBTA, a global open science Pediatric Brain Tumor Atlas initiative to comprehensively define the molecular landscape of pediatric brain tumors. The initiative contains multi-modal analyses of research- and clinical-trial based DNA and RNA sequences from nearly 1,000 subjects (with 1,256 tumors) along with their longitudinal clinical data. The OpenPBTA's open science framework for analysis tests the capacity of crowd-sourced collaborative architectures to advance more rapid, iterative and integrated discovery of the underlying mechanisms of disease across pediatric brain and spinal cord tumors. Since the launch of the project, OpenPBTA has collaboratively created reproducible workflows for integrated consensus SNV, CNV, and fusion calling, enabled RNA-Seq-based classification of medulloblastoma subtypes, and more than 25 additional DNA- and RNA-based analyses. The open-science platform and associated datasets and processed results provide a continuously updated, global view of the integrated cross-disease molecular landscape of pediatric brain tumors. Such biospecimen- and clinically-linked scalable data resources provide unprecedented collaborative opportunities for precision-based, personalized therapeutic discovery and drug development with the upcoming further integration of proteomic sample data (N >300) and drug response datasets, additionally diversifying the multimodal discovery potential of crowd-sourced approaches for accelerated impact for children with brain tumors.

RARE TUMORS/OTHER

RARE-01. ASSESSING THE SYMPTOM DIAGNOSTIC INTERVAL FOR CHILDREN WITH CENTRAL NERVOUS SYSTEM TUMOURS

Cameron Crowell[1,2], Bruce Crooks[1,2], Simon Walling[1,2], Kelly Boileau[1], Lynette Bowes[3,4], Robert Rutledge[5,2], Ketan Kulkarni[1,2], Daniel McNeely[1,2], and Craig Erker[1,2]; [1]IWK Health Centre, Halifax, Canada, [2]Dalhousie University, Halifax, Canada, [3]Janeway Children's Health Centre, St. John's, Canada, [4]Memorial University, St.John's, Canada, [5]Queen Elizabeth II Health Sciences Centre, Halifax, Canada

Background: Diagnostic delays in pediatric neuro-oncology is a subject of distress for families and providers. We aimed to evaluate the symptom diagnostic interval (SDI) and influencing variables for children with CNS tumors. Methods: This retrospective study analyzed 210 patients diagnosed from 2001–2018 and managed at the tertiary care facility in Halifax, Canada. SDI was defined as time from first symptom until tissue diagnosis or, if not available, imaging diagnosis. Non-parametric tests were used to compare SDI between groups. Results: Median SDI was 12.4 weeks (IQR 4.3–30), longer than 7 other studies of 1308 children reporting medians of 4.5–10 weeks ($p < 0.01$). Most common tumors and their median SDI included low-grade glioma (LGG) (n=97, 46%; 17.9 weeks), medulloblastoma (n=31, 15%; 8.7 weeks), high-grade glioma (HGG) and DIPG (n=23, 11%; 5.6 weeks), and ependymoma (n=13, 6%; 13.6 weeks). The most common initial reported symptom included headache (n=63; 30%), nausea/vomiting (n=27, 18%), seizure (n=24, 12%), and visual impairment (n=13, 6.3%). Patients aged 0–3 years had a shorter SDI than patients 10 years and older (SDI 8.7 vs 14.6 weeks; $p = 0.03$). Tumor category showed longer SDI for LGG versus HGG ($p = 0.003$), DIPG ($p = 0.02$), medulloblastoma ($p = 0.03$) and other embryonal tumors ($p = 0.03$). Longer SDI was not associated with increased risk of disease progression for LGG ($p = 0.93$), medulloblastoma ($p = 0.89$), or ependymoma ($p = 0.5$). No difference in SDI was found with regard to diagnosis era, ethnicity, socioeconomic status, or distance to the tertiary care facility. Conclusion: SDI at our centre is longer than previously reported studies. SDI is linked to tumor biology and its relevance within specific tumor groups deserves further investigation given it doesn't appear to predict tumor progression/recurrence, yet families and providers feel distress when delays in diagnosis are perceived.

RARE-02. POLYAMINE PATHWAY INHIBITION IS A POTENT NOVEL THERAPEUTIC STRATEGY AGAINST DIFFUSE INTRINSIC PONTINE GLIOMA

Aaminah Khan[1], Laura Gamble[1], Dannielle Upton[1], Denise Yu[1], Ruby Pandher[1], Chelsea Mayoh[1], Mark R. Burns[2], Murray D. Norris[1,3], Michelle Haber[1], Maria Tsoli[1], and David S. Ziegler[1,4]; [1]Children's Cancer Institute, Sydney, NSW, Australia, [2]Aminex Therapeutics, Inc., Kirkland, Washington, USA, [3]University of New South Wales Centre for Childhood Cancer Research, Sydney, NSW, Australia, [4]Kids Cancer Centre, Sydney Children's Hospital, Sydney, NSW, Australia

Diffuse intrinsic pontine glioma (DIPG) is an aggressive paediatric brainstem tumour, with a median survival of less than 1 year. Polyamines are intracellular polycations that control important aspects of cell growth and are often upregulated in cancer. Difluoromethylornithine (DFMO) is an FDA-approved inhibitor of the enzyme ornithine decarboxylase (ODC1) which is a key driver of polyamine synthesis. We investigated the efficacy of polyamine pathway inhibitors as a therapeutic strategy against DIPG.