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

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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

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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

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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)

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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-