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

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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

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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

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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

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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

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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-