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-

eral ophthalmologic side effects, including MEK inhibitor-associated retinopathy (MEKAR), also termed central serous-like retinopathy. MEKAR is defined as accumulation of subretinal fluid. It occurs in up to 90% of adults on MEK inhibitors, usually causing minimal to no symptoms, and typically resolving without MEK inhibitor dose adjustment. We report a case of MEKAR in a 15 year old boy with an optic pathway pilocytic astrocytoma with duplication of BRAF (7q34). Baseline ophthalmic exam showed 20/20 vision in his right eye with loss of the temporal hemifield and no light perception vision in the left eye. Nine months into treatment with Selumetinib his ophthalmologic exam and optical coherence tomography (OCT) showed asymptomatic bilateral subretinal fluid. Selumetinib was held for 2 weeks resulting in resolution of the subretinal fluid. Selumetib was resumed at the prior dose and MEKAR recurred 2 weeks later but then permanently resolved 4 weeks later despite remaining on Selumetib. Review of the literature discovered a single publication of 2 pediatric patients with optic pathway glioma who developed MEKAR around 6-7 months after initiating Selumetinib, which resolved after stopping Selumetinib. One patient was symptomatic and despite resolution of symptoms, Selumetinib was not resumed. The other patient was asymptomatic and resumed Selumetib, but redeveloped MEKAR 8 months after restarting Selumetinib. Based on adult experience and the limited pediatric experience outlined above, we recommend pediatric patients with asymptomatic and mild-moderate symptomatic MEKAR undergo close monitoring without Selumetinib dose interruption or modification unless symptoms progress.

LGG-64. A PHASE II STUDY OF PEGYLATED INTERFERON IN CHILDREN WITH RECURRENT OR REFRACTORY AND RADIOGRAPHICALLY OR CLINICALLY PROGRESSIVE JUVENILE PILOCYTIC ASTROCYTOMAS AND OPTIC PATHWAY GLIOMAS (NCT02343224)

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Unresectable Juvenile Pilocytic Astrocytomas (JPA) and Optic Pathway gliomas (OPG) are chronic diseases that can have solid +/- cystic components. We wanted to evaluate the objective response to pegylated interferon Alpha2B in this group of patients in a prospective single arm Phase II clinical trial. Eligibility criteria: age 3 -25 years, patients with neurofibromatosis (NF) were eligible, evidence of measurable disease in MRI, no limitation in the number of prior therapies including chemotherapy and radiation. Exclusion criteria: prior pegylated interferon exposure, less than 2 years from radiation, active autoimmune disease. Subjects enrolled received pegylated interferon 1 mcg/kg/dose SQ weekly, to a max dose of 150mcg/dose in 28 day cycles for up to 2 years. The study design is a Simon two stage design. If no complete or partial responses among the first 9 patients, the study will terminate. Nine subjects enrolled: 4 females, 5 males, median age of 11years, 6 Caucasians, 3 African Americans. Two subjects with NF. Molecular findings KIAA-BRAF fusion (6), V600E mutation (1), CDK2A loss (1). Location: brain (7), brain and spine (2). We enrolled a heavily pre-treated population, patients with prior radiation (1), nine with prior chemotherapy, the average number of regimens 4 (range 2-6). No complete responses or partial responses were seen. Two patients with prolonged stable disease 75+months and 66+ months. At 12 and 24 month EFS 76.2% (95%CI52.1-100%). Median EFS has not been reached. The 12 and 24 months survival estimate 75% (95% CI 50.3-100%), median survival has not been reached. Side effects as expected mostly grade 1-2. No grade 4 event related to pegylated interferon were seen. This is the first report of pegylated interferon in OPG and JPA, two patients with prolonged stable disease suggesting that pegylated interferon can offer potential benefit in this population and additional studies are important.

MEDULLOBLASTOMA

MEDB-01. MANAGEMENT OF THE RISK OF MEDULLOBLASTOMA ASSOCIATED TO FAMILIAL ADENOMATOUS POLYPOSIS AND DYSREGULATED WNT SIGNALOSOME

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APC is the key gene of the familial adenomatous polyposis (FAP). This tumor suppressor gene functions by negatively regulating the β-catenin protein and the majority of APC mutations disrupt the β-catenin degradation complex signalosome leading to the activation of the canonical Wnt pathway. Pathogenic APC mutations were reported in association to medulloblastoma. In this study, we report rare mutations of the APC gene detected in Tunisian families from the governorate of Sfax presenting clinically with various digestive and extra-digestive manifestations. Our goal was to assess the oncogenic risks encountered by our pediatric carriers to offer an accurate genetic counselling, particularly at the neurologic level. Molecular investigation of all members of two families was conducted, using bidirectional sequencing of all 15 exons of the APC gene. A phenotype-genotype correlation was conducted to elucidate the mutational pathophysiological mechanism. Two rare mutations were revealed in our familial study. The first mutation was located at exon 13 and was a missense mutation at codon 1690. The second mutation was a deletion identified at codon 4652 in exon 15. The mutations resulted both in truncated gene products. Clinical manifestations closely depending on the position of the mutation were respectively colic polyposis for the first mutation and soft tissue fibromatous tumors for the second. The localization of the APC mutations allows better targeting of surveillance for clinical manifestations that may be included in FAP. Mutations that remove the Axin-binding sites, as is the case for the first mutation, lead to severe clinical pictures whereas mutations that retain one or two of the Axin binding sites are associated with other features such as desmoid tumors. The risk of the Wnt- medulloblastoma subtype, higher among patients with FAP should be considered with a more awareness of signs and symptoms related to CNS tumors in the FAP context.

MEDB-02. THE IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF CIRCULAR RNA CIRC_63706 IN SONIC HEDGEHOG MEDULLOBLASTOMAS

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Circular RNAs (circRNAs) are increasingly recognized as stable and specific biomarkers and therapeutic targets in many cancers, but little is known about their function, subtype specificity, and biomarker potential in medulloblastomas. Medulloblastoma is a central nervous system tumor that predominantly affects children and always requires aggressive therapy. Understanding and identifying novel disease-related molecular mechanisms and pathways are essential for developing optimal and novel therapies. To identify medulloblastoma subgroup-specific circRNAs, we subjected RNA-seq data from 175 clinical medulloblastoma samples representing the four subgroups to a statistical and machine learning (random forest classification) pipeline. Circular RNA circ_63706 expression was specific to the sonic hedgehog (SSH) group, which was confirmed through *in situ* hybridization analysis of clinical tissue samples. Functional characterization of circ_63706 by siRNAs and shRNAs demonstrated that cell proliferation, invasion, and apoptosis are perturbed in circ_63706 cells and inhibited *in vivo* tumor growth. These novel medulloblastoma-specific circular RNAs are emerging as important oncogenes that not only provide valuable mechanistic insights into how medulloblastomas develop but also how they can be used as biomarkers and therapeutic targets. These results pave the way for the specific identification and personalized treatment of different medulloblastoma subgroups.

MEDB-03. MEDULLOBLASTOMA CEREBROSPINAL FLUID REVEALS HYPOXIC INDICATORS (METABOLITES AND LIPIDS) AND CANCER-SPECIFIC RNAS

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Medulloblastoma (MB) is the most common malignant brain tumor in children. There remains an unmet need for diagnostics to sensitively detect the disease, particularly recurrences. Cerebrospinal fluid (CSF) provides a window into the central nervous system, and liquid biopsy of CSF could provide a relatively non-invasive means for disease diagnosis. There has yet to be an integrated analysis of the transcriptomic, metabolomic, and lipidomic changes occurring in the CSF of children with MB. CSF samples from patients with (n=40) or without (n=11; no cancer) MB were subjected