RAF inhibitors target this pathway and are efficacious in early phase trials in recurrent pLGGs. However, not all patients respond to monotherapy, and many experience progression after completion of therapy. Evaluating combination therapies that may enhance efficacy or prolong disease stabilization is warranted. Lenalidomide is an immunomodulatory agent with an anti-tumor effect demonstrated in phase 1 trials in recurrent pediatric central nervous system (CNS) tumors. OBJECTIVE: To describe our institutional experience using concurrent trametinib and lenalidomide in the treatment of primary pediatric central and peripheral nervous system (PNS) tumors. METHODS: Retrospective review of patients' medical records. RESULTS: Four patients with locally recurrent primary CNS or PNS tumors, three with WHO grade II pilomyxoid astrocytomas and one with a plexiform neurofibroma, were treated with trametinib and lenalidomide concurrently. Two patients developed severe thromboembolic events. One patient was treated with combination therapy for seven months until trametinib and lenalidomide were held after urgent ventriculoperitoneal shunt revision. Shortly following shunt revision, he experienced near-complete vision loss. MRI of the brain demonstrated a left posterior watershed territory hypoxic-ischemic injury. In a second patient, after four months of combination therapy, surveillance echocardiogram showed an incidental finding of severe biventricular dysfunction with a left ventricular ejection fraction (LVEF) of 17.7% and two mural thrombi in the left ventricular apex. She started losartan and enoxaparin and discontinued trametinib and lenalidomide. Her LVEF normalized four months later, and the mural thrombi resolved. CONCLUSIONS: Given the severe thromboembolic events experienced by these patients treated with concomitant trametinib and lenalidomide, this combination requires further investigation, and we urge caution if used concurrently.

LGG-43. REDUCTION IN THE CEREBROSPINAL FLUID PROTEIN LEVEL AFTER BEVACIZUMAB TREATMENT IN PATIENTS WITH OPTIC PATHWAY LOW-GRADE GLIOMAS

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Cases Presentations: Case 1: A 1-year-old boy with suprasellar pilocytic astrocytoma with previous history of shunting disfunction, treatment according vinblastine protocol due to anaphylactic reaction with carboplatin presented with ascites and necessity of ventricular-atrial shunt. Due to high protein cerebrospinal fluid (CSF) level (551mg/dl) he was submitted to external ventricular drainage and bevacizumab 10mg/kg was associated to his oncology treatment. After three cycles of bevacizumab, the patients' CSF protein levels decreased dramatically 178 mg/dL, allowing the shunt procedure without complications and shorter hospital stay. Case 2: A ten-year-old boy with suprasellar pilocytic astrocytoma treated with three lines of chemotherapy showed tumor progression one year after the end of carboplatin-vincristine protocol and shunting disfunction. External ventricular drainage was performed, and the CSF showed 590mg/dl protein level. He was treated with vinblastine 6mg/m2 weekly and bevacizumab 10mg/kg each 14 days. After two cycles of bevacizumab, the protein level was 191mg/dl allowing another V-P shunt procedure. Discussion: Optic pathway gliomas frequently cause elevated cerebrospinal fluid protein concentrations leading to shunts occlusions and failures, necessity of external ventricular drainage and longtime hospitalization, implicating risk of serious infections. Bevacizumab is a monoclonal antibody with immunomodulatory and anti-vascular endothelial growth factor (VEGF) activities that has been used in combination with other chemotherapeutic agents such as irinotecan and vinblastine to treat low-grade gliomas and has been reported to decrease the CSF protein concentration.Final Comments: Bevacizumab treatment in patients with gliomas and high CSF protein levels seems effective in decreasing protein leakage from the vessels to the ventricles, thereby improving the scope for successful shunt placement.

LGG-44. MULTI-OMIC ANALYSIS REVEALS INTEGRATED SIGNALLING NETWORKS IN PAEDIATRIC LOW-GRADE GLIOMA

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Paediatric low-grade gliomas (pLGGs) are the most common type of childhood CNS tumours. Our study included pilocytic astrocytomas (PAs; KIAA1549:BRAF), glioneuronal tumours (GNTs; BRAFV600E) and location-matched controls. We initially performed kinase substrate enrichment analysis (KSEA) to infer differential kinase activity, which allowed us to identify altered signalling networks in the two tumour types. Here we report the integration of these kinase signalling networks together with total proteomics, transcription factor enrichment analysis (TFEA) and transcriptomics (coding and non-coding). Total proteomic profiling confirmed an increase in proteins involved in cell cycle, inflammatory response and signal transduction in PAs, whilst there was an increase in proteins promoting cell growth, immune response and inflammation in GNTs. TFEA was performed using the DoRothEA database to identify master transcriptional regulators. We observed significant activation of transcription factors (TFs) that are direct targets of MAPK signalling in both tumour types. Notable differences include the higher activation of NF-kB/STAT TFs in PAs and the increased activation of RFX1/2 in GNTs. Next, we constructed kinase-TF networks and identified multiple kinases targeting STAT3 in PAs and STAT1/3 in GNTs. Pathway analysis of RNA-Sequencing data showed enrichment of NF-kB in both tumours and repression of E2F target genes (PA) and reduced expression of MYC target genes (GNT). We developed a BRAF-OIS signature and found 23 genes commonly enriched in both tumour types, highlighting shared senescence-associated targets. MicroRNA profiling identified upregulation of microRNAs that target MAPK and NF-kB signalling networks, and many down-regulated microRNAs with tumour suppressive roles. Finally, we identified several lncRNAs known to be differentially expressed in glioma and, whilst their mechanism(s) of action are varied, they are thought to act with other well-established regulators to fine-tune cellular processes. Taken together, we present a comprehensive signalling network as a framework for studying pLGGs.

LGG-45. GENETIC DEPENDENCIES IN *MYB/MYBL1*-DRIVEN PEDIATRIC LOW-GRADE GLIOMA MODELS

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AIM: Pediatric low-grade gliomas (pLGGs) are a heterogenous group of tumors, diverse in their localization, histology, mutational landscape, clinical behavior, and treatment response. Genomic alterations impacting the MYB family of transcription factors were identified in two distinct pLGG subtypes: Angiocentric Gliomas (AG) and Diffuse Astrocytomas (DA). The molecular profiles and therapeutic vulnerabilities associated with these genomic alterations remain unexplored. In this study we highlight the use of genome-wide CRISPR/Cas9 knock-out screens for an unbiased identification of translatable therapeutic targets. **METHODOLOGY**: Given the lack of patient-derived pLGG cell lines, we engineered *in vitro* pLGG mouse and human neural stem cell (NSC) models to harbor pLGG-relevant genomic alterations. We performed single cell RNA sequencing to investigate the transcriptional profiles driven by these mutations and to dissect the central regulatory networks enabling tumorigenesis. Specific genetic dependencies associated with *MYB/MYBL1* mutations were screened using the Brie genome-wide mouse CRISPR lentiviral knock-out pooled library, consisting of 78,637 single guide RNAs (sgRNAs) targeting 19,674 mouse genes. RESULTS: We have successfully generated *in vitro* NSC-based pLGG models crucial to deepening our knowledge on pLGG biology and the identification of translatable therapeutic targets. Genome-scale CRISPR/Cas9 knock-out screens in isogenic NSCs models, expressing distinct *MYB/MYBL1* alterations or a control transgene, revealed several differential genetic dependencies. Among the top identified dependencies are regulators of cell-stress response, cell-cycle progression, and modulators of the ubiquitin-proteasome degradation pathway. **CONCLUSION**: Genome-wide CRISPR knock-out screens are a powerful tool for the unbiased identification of mutation-specific genetic dependencies that can be explored as candidates for precision medicine approaches.

LGG-46. SURVIVAL OF THE FITTEST? A PROGNOSTIC EVALUATION OF PAEDIATRIC LOW-GRADE GLIOMA (PLGG) SURVIVOR FUNCTIONAL OUTCOMES

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INTRODUCTION: Whilst most patients with PLGG will survive, varying morbidities derived from patient,tumour & treatment characteristics can afflict life-long disabling functional impairments. No PLGG studies have evaluated potential prognostic factors for important functional outcomes. METHODS: We performed retrospective analysis of all children diagnosed with PLGG at GOSH 1980-2021.Review of medical notes recorded patient demographics,tumour characteristics & treatment data.Functional outcomes included endocrine,educational,visual (in OPG),auditory & physical function.Multivariate regression analysis($p<0.05$) examined associations between biological prognostic variables & functional outcomes. RESULTS: 814 patients were diagnosed with PLGG.731(90%) had 5-years follow-up from diagnosis & were included for functional analysis.Median age at diagnosis 7 years(0-17.9); 50.6%Male,12.2% NF1.Tumours were cerebral(27%),cerebellar(27%),hypothalamo-chiasmatic(19%),brainstem(7%),or other(20%);with disseminated disease in 5%.Pilocytic Astrocytoma constituted 46%.Molecular profiling of 133 revealed *5%BRAFV600E mutation*,42%*BRAF-KIAA1549* fusion.Treatments included: Surgery(70%),Chemotherapy(20%),& Radiotherapy(21%).20-year-OS 94%,PFS 76%;median follow-up 16 years(5-38). Documented neurocognitive deficiency(30%) associated with chemotherapy(HR2.36,95%CI 1.49-3.75,P<0.001), radiotherapy(HR 2.25,95%CI1.5-3.36,P<0.001) & male gender(HR 0.68,95%CI 0.49-0.95,P0.02)as independent poor-prognostic risk-factors.Chemotherapy(HR 5.7,95%CI1.4-22.3,P0.01) & radiotherapy(HR6.77,95%CI2.1-22.0,P0.001) were independent risk-factors for requirement of Educational-Health-Care-Plans(25%).9% attended specialised schools. Combined-limb-MRC-grade <18/20(6.4%) was independently-associated with receiving chemotherapy(HR 2.77,95%CI1.29-5.93,P0.01),& radiotherapy(HR 6.28,95%CI3.25-12.15,P<0.001).6% mobilised by wheelchair.Resolution of seizures occurred in 68% of 176 following PLGG treatment. Single/multiple endocrinopathies occurred in 9.3%/11%.Presence of 2+Endocrinopathies was associated with chemotherapy(HR6.82,95%CI4.0-14.4,P<0.001), radiotherapy(HR7.81,95%CI4.3-14.3,P<0.001),NF1(HR2.9,95%CI1.3-6,P0.01),OPGs(HR 1.3,95%CI1.2-1.5,P<0.001);with younger diagnostic-age(HR0.80,95%CI0.74-0.87,P<0.001) & initial surgical resection(HR0.3,95%CI0.15-0.7,P0.03) independent protective factors. Receiving chemotherapy/radiotherapy were independent prognostic-factors for Post-PLGG-treatment Brock grade 1+ hearing impairments(2.2%).Visual outcomes in 146 OPG patients:blindness in atleast 1 eye(4.8%),registered visual impairment(9.6%),& visual-aid use(6.2%). **CONCLUSIONS**: Whilst overall outcomes for PLGG are optimistic, some patients have significant functional impairments detrimental to quality-of-life.Further evaluation of longer-term functional outcomes and prognostic associations is justified.

LGG-47. SINGLE-CELL RNA SEQUENCING REVEALS IMMUNOSUPPRESSIVE MYELOID CELL DIVERSITY DURING MALIGNANT PROGRESSION IN GLIOMA

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Myeloid cells and macrophages have been shown to promote immunosuppression in high-grade gliomas (HGG), however their roles in malignant progression of low-grade glioma (LGG) are poorly understood. Here, we investigated the heterogeneity of the immune microenvironment during glioma progression using a murine model that recapitulates the malignant progression of low to high-grade glioma. To that end, we performed single-cell RNA sequencing on CD45+ immune cells isolated from animals bearing no tumor (NT), LGG, and HGG. We observed an increased infiltration of CD4+ T cells, CD8+ T cells, B cells, and natural killer cells in the tumor microenvironment of LGG, whereas this infiltration was abrogated in HGG. Our study identified two distinct macrophage clusters across all 3 samples, with signatures of bone marrow derived and resident macrophages, respectively. These macrophages showed an immune-activated phenotype *(Stat1, Tnf, Cxcl9 and Cxcl10)* in LGG, but then evolved to a more immunosuppressive state (*Lgals3, Apoc1 and Id2*) in HGG, restricting T cell recruitment and activation. In addition, we identified CD74 and macrophage migration inhibition factor (MIF) as potential targets for both these distinct macrophage populations, based on their increased expression in LGG and HGG compared to NT. Targeting these factors during the LGG therapeutic window may inhibit myeloid cells and intra-tumoral macrophages and attenuate their immunosuppressive properties and impair malignant progression.

LGG-48. THE INFLUENCE OF DIFFERENT FGFR1 ALTERATIONS ON PEDIATRIC LOW-GRADE GLIOMA TUMOR BIOLOGY AND TARGETED THERAPY RESPONSE

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Pediatric low-grade gliomas (pLGGs) have excellent survival, however, with current standard of care, most patients suffer lifelong severe sequalae. pLGGs are almost exclusively driven by single activating mutations in the MAPK pathway. Clinical trials with small molecule inhibitors in BRAF-altered pLGGs are showing promising results in early clinical trials, and similar efforts are now underway for FGFR1-altered tumors, however the underlying biology and treatment response has not been thoroughly explored in a pre-clinical setting. To explore the genetic landscape of FGFR altered gliomas we assembled a cohort of 87 patients with FGFR1-4 altered gliomas across Dana-Farber Cancer Institute, Boston Children's Hospital and Brigham and Women's Hospital. Within this cohort we observed that pLGGs harboring FGFR1 kinase hotspot mutations (FGFR1-N546K or -K656E) frequently harbored a second alteration associated with activation of the MAPK or mTOR pathways, most commonly in the phosphatase PTPN11, NF1 or within the FGFR1 gene itself. Additionally, we observed two previously described structural variants of FGFR1, an FGFR1 internal kinase tandem duplication (FGFR-ITD) and a fusion with TACC1 (FGFR1:TACC1). The relative impact of the different FGFR1 alterations on oncogenicity, therapeutic response and resistance has not been previously explored. To address this, we have established mouse neural stem cell models overexpressing the structural variants and hot spot mutant FGFR1 alone or in combination with a second alteration. Immunoblotting revealed that the addition of a second alteration attenuated phosphorylation of ERK, AKT and S6 and influenced cell proliferation both in normal growth conditions and in absence of growth factor. Treatment with inhibitors of FGFR (Infigratinib) and MEK (Trametinib) revealed variable sensitivity both targeted therapies, suggesting that treatment of FGFR1 driven pLGG might require tailoring to the specific FGFR1 alteration.

LGG-49. SUBEPENDYMAL GIANT CELL ASTROCYTOMA ASSOCIATED WITH A CORTICAL TUBER: A CASE REPORT

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Subependymal giant cell astrocytomas (SEGAs) are circumscribed gliomas strongly associated with tuberous sclerosis (TS). TS, a rare genetic disorder caused by inactivating mutations in either of the TSC genes (TSC1/2), leads to upregulation of the mTOR pathway and consecutive cell growth. CNS manifestations other than SEGAs include cortical tubers, white matter glioneuronal hamartomas and subependymal nodules (SENs), which, although regarded as distinct morphological phenotypes, share certain histological characteristics including ballooned astrocytes and giant ganglion-like cells. SEGAs, thought to develop from the median ganglionic eminence (MGE), are most commonly located periventricularly. However, rare cases of extraventricular SEGAs have been reported. We report a case of a cortico-subcortically located SEGA in a TS patient. A two-month-old female TS patient with multiple cortical tubers presented with treatment-resistant epilepsy. A 4cm sized tuber located in the right temporal lobe, showing a transmantle sign on MRI but no typical imaging appearance of SEGA, was surgically resected. Histological evaluation revealed typical morphological characteristics of a tuber with dysmorphic neurons, balloon cells and calcifications in the cortex and adjacent white matter. Focally, a cortico-subcortically located, well delineated area with increased cellularity and morphological features of a SEGA was found. Single mitotic figures were detectable. Immunhistochemically, these cells were strongly positive for GFAP, vimentin, and nestin. Scattered S100-, NeuN-, MAP2- and SMI32-positive cells were present. No expression of class-III-b-tubulin and TTF1a was found. pS6 was expressed in a small fraction of cells. CD34 showed a dense capillary network within the lesion. This case is consistent with prior case reports of SEGA-tissue in tubers of TS patients. However, the SEGA tissue of this case did not display TTF1a-expression, characteristic for SEGAs and considered as a marker for cells originating from the MGE, thus implying a different cellular lineage.