MAPK-related signatures were downregulated upon MEKi treatment, while pathways related to upstream MAPK activators (including FGFR, NTRK and TGFB pathways) were upregulated, in both proliferating and senescent DKFZ-BT66. Genes regulated by the MAPK pathway and involved in OIS-SASP were identified by analyzing genes differentially regulated between proliferating and senescent DKFZ-BT66, and modulated upon MEKi treatment. Conclusion: This data suggests that MAPKi reverses OIS in senescent PA cells, while inducing the activation of MAPK upstream regulators in proliferating and senescent PA cells, identifying putative co-targets that could help prevent growth rebound upon MAPKi withdrawal. Furthermore, the identification of the MAPK-related OIS-SASP genes provide insight about the regulation of OIS-SASP by the MAPK pathway. Validation of this data with the ongoing phospho-proteomic analysis and in primary samples is needed.

LGG-05. GENERATION OF NOVEL MOUSE MODELS FOR BRAF V600E MUTANT GLIOMAGENESIS TO GAIN MECHANISTIC INSIGHTS INTO TUMOR FORMATION AND PROGRESSION

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Background: The BRAF V600E mutation occurs in ~ twenty percent of histologically diverse pediatric gliomas and is the second most common mutation in pediatric low-grade gliomas (LGG). BRAF V600E expression in LGG with balanced CDKN2A is associated with a higher rate for progression than for BRAF V600E wildtype tumors, and despite adjuvant therapy, consisting of resection, radiation and chemotherapy. Progression invariably occurs in BRAF V600E mutant CDKN2A deleted gliomas, marking a high-risk group. Here, we aim to overcome the lack BRAF V600E mutant glioma models that allow for studies of stem and progenitor cells and the immune system ability to understand progression. Methods: We develop novel immunocompetent, stem and progenitor cell-based mouse models for BRAF mutant gliomas, including genetically engineered mouse models (GEMMs), orthotopic glioma models derived from gliomas in GEMMs as well as in vitro models of those tumors. BRAF mutant mouse brains and cells were analyzed by immunofluorescence staining, flow cytometry, mass cytometry and RNA sequencing. Results: Ongoing model development studies indicate that BRAF V600E mutant gliomas in murine brain exhibit very similar neuroanatomical preferences to human gliomas. The BRAF V600E mutation exacerbates the heterogenous cell cycling pattern of normal neural stem and progenitors and expands a symmetrically dividing progenitor population. Cellular plasticity rather than cellular lineage hierarchy drives the generation of a therapy resistant stem cell pool. Transcriptomic analyses of neuroglial stem cells with induced BRAF V600E expression provide insights into mechanisms for neoplastic transformation and progression. Conclusion: Analyses of two independent BRAF V600E mutant mouse models provide novel insights into the role for tumor intrinsic factors, such as plasticity and stemness, and the tumor microenvironment in progression.

LGG-06. COMPREHENSIVE GENOMIC CHARACTERIZATION AND INTEGRATED CLINICAL ANALYSIS OF LOW-GRADE GLIOMAS IN CHILDREN WITH NEUROFIBROMATOSIS TYPE 1

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Background: Low-grade gliomas (LGGs) arising in children with neurofibromatosis type 1 (NF1) are usually not biopsied. To identify secondary genetic alterations or molecular features that may contribute to pathogenesis and correlate with clinical behavior, we initiated a comprehensive molecular and clinical analysis of pediatric NF1-LGGs. Methods: NF1-LGGs were analysed by whole-genome sequencing (31), targeted gene panel sequencing (9), RNAseq transcriptomal profiling (33) and genome-wide DNA methylation analysis (67). Clinical annotation was available for 48 subjects. Results: Most LGGs harbored bi-allelic *NF1* inactivation as the sole genetic abnormality, but 11% had additional alterations (*FGFR1* mutation, n=3; *PIK3CA* mutation, n=2; homozygous 9p21 deletion, n=2; *MYB:QKI* fusion, n=1; *SETD2* mutation, n=1; *EGFR* amplification, n=1). *FGFR1* mutation conferred additional growth advantage in multiple complementary murine *Nf1* models. 88% of NF1-LGGs resembled sporadic pilocytic astrocytoma (PA) by methylation, higher than that based on histology. Non-PA methylation patterns included low-grade glial/glioneuronal tumors, rosette-forming glioneuronal tumors, MYB/MYBL1-altered glioma, and high-grade astrocytoma with piloid features (2 tumors histologically diagnosed as LGG). In total, 18% of samples were classified as non-PA and/or harbored an additional non-*NF1* mutation. Non-PA methylation class tumors were more likely to harbor an additional non-*NF1* mutation (p=0.005). 7.7% of optic pathway hypothalamic gliomas (OPHGs) had other mutations or were not classified by methylation as PA, compared with 20.6% of NF1-LGGs arising elsewhere. There was no difference based on age for the presence of an additional non-*NF1* mutation or non-PA methylation class. Conclusions: Given the overall low occurrence of non-*NF1* mutations or non-PA methylation class tumors in this series, routine clinical biopsy of typically-appearing NF1-LGG may not be indicated, particularly for children with OPHG. Biopsy should be considered for non-OPHG tumors refractory to conventional treatment. As additional agents are developed and treatment strategies evolve, the rationale for biopsy of NF1-LGG may become stronger.

LGG-07. IS BRAF ALTERATION OR A HISTOLOGIC 'QUALIFIER' A PREDICTOR OF OUTCOME IN PEDIATRIC PILOCYTIC ASTROCYTOMA?

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Introduction: Pediatric pilocytic astrocytomas (PA) are the most common pediatric central nervous system tumor. Surgical resection is the primary treatment for PA with five-year survival rates up to 95%. Despite a favorable prognosis, our understanding about the prognostic value of histopathological findings, such as histopathologic qualifier* or *BRAF* alterations is evolving. Methods: Patients treated for a WHO grade 1 PA at Washington University in St. Louis/St. Louis Children's Hospital were analyzed for clinical details, including pathology diagnosis (*histopathologic qualifier refers to designations in the diagnosis such as "WHO Grade I pilocytic astrocytoma with increased proliferative index"). BRAF alterations include gene fusions and point mutations. Results: 224 patients were analyzed (51% female, mean age 9.6 years). Tumors were located in the cerebellum/fourth ventricle (50%), optic pathway/hypothalamus (15%), brainstem (12%), and cerebral cortex (11%). BRAF alterations were identified in 55/77 patients (71.4%) and additional histopathologic qualifiers were present in 27/220 patients (12.3%). 196 patients (87.5%) underwent surgical treatment and 22 (9.8%) had biopsy alone. 45 patients (22%) displayed tumor progression or recurrence after resection. The presence of a histopathologic 'qualifier' in the topline or *BRAF* alteration was not associated with tumor progression or recurrence (p=0.36, p=0.77). Ki-67 proliferative indices were not predictive of progression or recurrence (p=0.94), including when controlling for extent of resection and adjuvant therapy. *BRAF* alterations, specifically *KIAA1549* fusions, were associated with cerebellar/fourth ventricular tumor location (p<0.001) and younger patient age (p=0.03). Extent of resection was the only predictor of outcome identified in this study; patients with gross total resection had significantly lower rates of progression and recurrence (p<0.0001). Conclusion: *BRAF* alterations and histopathologic qualifiers were not associated with tumor progression or recurrence in pediatric PA, although *BRAF* fusions were more common in tumors located in the cerebellum/fourth ventricle and in younger patients.

LGG-08. TREATMENT OUTCOMES AND TOLERABILITY OF TRAMETINIB IN PROGRESSIVE CIRCUMSCRIBED LOW-GRADE GLIOMAS

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Circumscribed low-grade gliomas comprise roughly one-third of pediatric CNS tumors. Most of these tumors are caused by activating mutations in the mitogen-activated protein kinase (MAPK) pathway. Drugs targeting the MAPK pathway are effective in other cancers and are being utilized in low-grade gliomas. We describe treatment outcomes and toxicities in a series of thirteen low-grade glioma patients treated with trametinib. We performed a retrospective chart review to evaluate response on T2/FLAIR MRI images per updated RANO criteria, visual outcomes, tolerability, and durability of response in progressive low-grade glioma patients treated with trametinib. Thirteen patients age 22 months to 34 years were included. Best radiographic response on therapy included 2/13 partial response, 3/13 minimal response, 5/13 stable disease, and 3/13 progressive disease. Diagnoses included pilocytic astrocytoma (n=6), desmoplastic infantile ganglioglioma (DIG; n=1), and low-grade glial neoplasms (n=2). Molecular drivers included BRAF:KIAA1549 fusion (n=3), V600E mutation (n=1), and somatic NF1 mutation (n=1). Three patients had germline NF1. In patients with partial or minimal response, best response was seen after longer durations of therapy; 4 of 5 best responses occurred after at least 12 months on therapy. Five patients completed prescribed therapy. Three patients remain stable off therapy at 6, 12, and 21 months; two patients recurred at 1 and 10 months off therapy. Skin manifestations were the predominant form of toxicity. This was more severe in older males, and symptoms improved with intermittent dosing. All patients with optic pathway tumors showed at least stable vision throughout treatment, with some patients having dramatic improvement. Trametinib is effective and well-tolerated in patients with low-grade glioma. Dermatologic toxicity can be mitigated by intermittent dosing. Best responses tended to occur later in therapy, sometimes after relatively stable MRIs. Patients with optic pathway lesions showed stable to improved vision even in the absence of significant radiographic response.

LGG-09. SENOLYTIC AGENT NAVITOCLAX TARGETS VINBLASTINE- AND MAPK INHIBITORS-INDUCED SENESCENT TUMOUR CELLS IN PAEDIATRIC LOW GRADE GLIOMAS

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Pilocytic astrocytoma (PA, WHO grade I), the most common paediatric brain tumour, is characterized by constitutive activation of the MAPK pathway. PA tumours show a slow growth, without tendency to progress to high-grade malignancies. However, a significant group of patients for whom a total resection is not feasible require additional therapy. The typical proliferative index of a PA, measured by Ki-67 staining, is 1–2%, whereas a large part of the tumour is Ki-67 negative and expresses markers of oncogene-induced senescence (OIS) such as SA-β-Gal positivity and the cell cycle inhibitors $p16^{INK4a}$ (CDKN2A) and $p21^{Cip1}$ (CDKN1A). Conventional treatments (i.e. chemotherapy) tend to target only proliferative cells and the effect of new molecularly targeted therapies (e.g., MAPK pathway inhibitors) on senescent cells remains unclear. Here, we discuss the opportunities to combine these therapies with new compounds targeting the senescent cells, referred to as senolytics, using three different PA models. (1) *Ex vivo* culture of human PA tumours (2) Two cell lines: the DKFZ-BT66 PA human cell line, carrying the oncogenic driver KIAA1549:BRAF-fusion, used as a model of OIS; and the proliferative BT40 cell line harbouring the $BRAF^{V600E}$ mutation; (3) *In vivo* xenograft model induced by orthotopic transplantation of BT40 cells. We have previously shown that OIS cells exhibit an increased sensitivity to senolytic compounds, such as navitoclax, a clinically approved BCL2/XL inhibitor, relative to proliferative controls *(Buhl et al, Clin Cancer Res. 2019)*. Our current research demonstrates that treatments with low doses of chemotherapy (e.g., vinblastine) or MAPK inhibitors (e.g., dabrafenib or trametinib) triggers a therapy-induced senescence response in proliferative cells (e.g., abolished proliferation, SA-β-Gal positivity, SASP production), making these senescent cells sensitive to senolytic compounds, including navitoclax. Together, our research provides a strong rationale supporting the combined use of senolytics with current conventional and targeted therapies against human PA.

LGG-10. AN UNUSUAL PRESENTATION OF BILATERAL OPTIC NERVE GLIOMA IN CROUZON SYNDROME

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Optic pathway gliomas (OPGs) are low grade gliomas intrinsic to the visual pathway, frequently associated with Neurofibromatosis Type 1 (NF-1). Bilateral OPGs without chiasmatic involvement are almost pathognomonic for NF-1. We report an unusual case of bilateral optic nerve glioma without chiasmatic involvement in a 17-month old male patient with craniosynostosis and Crouzon Syndrome, an autosomal dominant disorder caused by activating *FGFR2* mutations associated with craniosynostosis and optic atrophy. The patient's c.1032 G>A pathogenic variant in *FGFR2* is a variant known to affect splicing and results in a protein that lacks part of an important domain involved in ligand binding. Although *FGFR1* mutations have been implicated in low-grade glioma through MAPK activation, *FGFR2* mutations have not yet been described in OPGs, although they have been described in epileptogenic low-grade gliomas and mixed neuronal-glial tumors. Our patient presented with worsening vision in the setting of known Crouzon Syndrome. An eye examination revealed bilateral primary optic atrophy. Brain and orbital MRIs demonstrated fusiform dilation and STIR hyperintensity of the intraorbital segments of the optic nerves bilaterally with normal pre-chiasmatic optic segments. There were no other radiographic or physical stigmata suggestive of NF-1. Next generation sequencing and copy number analysis from peripheral blood were negative for variants in *NF1*. RNA based studies for *NF1* aberrations are pending. Although follow up MRI scans demonstrated stable size of his OPGs, the risk of further visual deficit was considered significant due to his pre-existing optic atrophy and poor baseline visual acuity. Therefore, he was started on vincristine and carboplatin chemotherapy according to A9952, and induction therapy has been well tolerated. To our knowledge, this is the first patient with Crouzon Syndrome who has developed bilateral optic pathway gliomas. Orbital MRIs should be considered for these patients with worsening visual acuity not explained by other causes.

LGG-11. BH3-MIMETICS TARGETING BCL-XL SELECTIVELY IMPACT THE SENESCENT COMPARTMENT OF PILOCYTIC ASTROCYTOMA

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Introduction: Pilocytic astrocytoma (PA) is the most common brain tumor in children. Activation of the mitogen-activated protein kinase (MAPK) pathway is a hallmark of PA. Complete remission in non-resectable tumors is infrequently observed with current therapeutic approaches. Most PA tumors cells are in oncogene-induced senescence (OIS), which may explain the benign growth behavior of PAs but also account for resistance to therapy. Therefore, treatment of PA with senolytic agents such as BH3-mimetics is a promising new approach. Methods: Three patient-derived PA cell lines, DKFZ-BT66, DKFZ-BT308 (both KIAA1549:BRAF-fusion positive) and DKFZ-BT314 (BRAF V600E-mutation positive) were used. Depending on inducible expression or repression of SV40 large T antigen all models can reflect both states of PA, proliferation and OIS.