eration, migration and invasion of pHGG cell lines and resulted in cell cycle arrest. Furthermore, CLIC1 and CLIC4 deficiency exacerbated the killing capacity of TT fields. Whole transcriptome gene expression analysis (Human Clairom™ Array) of paediatric GBM cell lines treated with tumour treating fields and found that cells treated with TTfields exhibited a down-regulation in CLIC1 and CLIC4 compared to untreated cells. These data provide rationale that genetic, electrical, and pharmacological manipulation of ion channels will reduce the capacity of childhood brain tumours to proliferate and invade. Therefore, may be a suitable target for combination therapy to enhance the treatment efficacy of TTfields and help bring this non-invasive therapy to paediatric patients.

HGG-29. HOW I TREAT RECURRENT PEDIATRIC HIGH-GRADE GLIOMA (HGG): A EUROPE-WIDE SURVEY STUDY.

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PURPOSE: Prognosis of pediatric high-grade gliomas (pedHGG) is dismal, and there is no standard of care treatment in case of recurrence/progression. We aimed to gain an overview of different treatment strategies in the setting of recurrent/progressing pedHGG. METHODS: In a web-based questionnaire, members of the SIOPE-BTG and the GPOH were surveyed on different therapeutic options in four real-world case scenarios (children/adolescents with recurrent/progressing HGG). RESULTS: One hundred and thirty-nine clinicians with experience in pediatric neuro-oncology from 22 European countries participated in the survey. Most respondents preferred further active (multimodal) oncological treatment in three out of four cases and chose palliative care with pure symptom control measures only in one case (gliomatosis cerebri and marked symptoms). Depending on the case, 8-92% of experts would initiate a re-resection (maximal safe resection in case of localized hemispheric pedHGG), combined with molecular diagnostics, and 55-77% recommended (re-)irradiation, preferably local radiotherapy >20 Gy (or craniospinal irradiation in one case with disseminated spinal HGG, 65%). Throughout all case scenarios, most respondents would participate in clinical trials and use targeted therapy (79-99%), depending on molecular genetic findings (BRAF alterations: BRAF/MEK inhibitors, 64-88%; EGFR overexpression: anti-EGFR treatment, 46%; SMARCB1 deletion: EZH2 inhibitor, 12%). 31-72% would administer chemotherapy (CCNU, 17%; PCV, 8%; temozolomide, 19%; oral etoposide/trofosfamide, 8%), and 20-69% advocated immunotherapy (checkpoint inhibitors, 30%; tumor vaccines, 16%). Depending on the individual case, respondents would also include bevacizumab (6-18%), HDAC inhibitors (4-15%), tumor-treating fields (1-26%), and intraventricular chemotherapy (4-24%). CONCLUSIONS: In each case, experts would combine conventional multimodal treatment concepts, including re-irradiation, with targeted therapy based on molecular genetic findings. International cooperative trials combining a standard (chemo-)therapy backbone with targeted therapy approaches for defined subgroups may help to gain valid clinical data and improve treatment in pediatric patients with recurrent/progressive HGG.

HGG-30. H3.3 G34R/V MUTATIONS DISRUPT H3.3 MITOTIC PHOSPHORYLATION LEADING TO HIGH-GRADE GLIOMA FORMATION THROUGH THE INDUCTION OF CHROMOSOMAL INSTABILITY

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Pediatric high-grade gliomas (pHGG) are among the most lethal of all human cancers. Histone H3.3 G34R/V mutations are an early event in these tumors and show reduced H3.3 K36 trimethylation; implicating epigenetic dysregulation in tumorigenesis. Here we present evidence that H3.3 G34R/V mutations promote tumor formation via the induction of chromosomal instability (CIN). Pericentromeric H3.3 is phosphorylated at S31 by Chk1 during mitosis. We observed that the H3.3 G34R mutation reduced Chk1 phosphorylation of H3.3 S31 by >90% in vitro. Furthermore, H3.3 G34 mutant cells have reduced pericentromeric H3.3 S31 phosphorylation in mitosis compared to WT H3.3 cell lines. H3.3 G34 mutant pHGG cells also have significantly elevated rates of CIN as compared to H3.3 WT cells. Overexpression of H3.3 G34R, G34V or non-phosphorylatable S31A in H3.3 WT, diploid cells caused a significant increase in CIN, but H3.3 K36M overexpression had no effect on chromosome segregation. These studies demonstrate that H3.3 G34R/V mutations are sufficient to induce CIN in normal, diploid cells. To determine if this process contributes to tumorigenesis, we used RCAS Nestin-TVA mice to overexpress H3.3 WT, G34R, or S31A – P2A-linked to PDGFB in glial precursor cells of newborn mice. Over 100 days, S31A and G34R mice had drastically reduced survival (averaging 77, 81, and 100 days for S31A, G34R, and WT). Furthermore, most G34R and S31A mice developed HGG, while H3.3 WT mice remained tumor-free. Our work implicates CIN as a driver of H3.3 G34 mutant pHGG formation.

HGG-31. UNIQUE CASE OF A BITHALAMIC H3K27-WILDTYPE DIFFUSE MIDLINE GLIOMA, EGFR-ALTERED WITH METHYLATED MGMT

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BACKGROUND: Diffuse midline gliomas are aggressive pediatric brain tumors frequently associated with somatic mutations in histone genes *H3F3A* (H3.3) and *HIST1H3B* (H3.1), which promote gliomagenesis through reprograming of the epigenetic landscape by inhibiting the tri-methylation of H3K27 (H3K27-me3). H3K27M-mutant gliomas comprise over 80% of diffuse midline gliomas, and are characterized by dismal outcomes as well as near-ubiquitous absence of MGMT promoter methylation. The subset of H3K27-wildtype diffuse midline gliomas remains incompletely understood with regards to underlying pathogenesis, therapeutic targets, and prognosis. We present the clinical, imaging, histopathologic, and molecular characteristics of a pediatric patient with a bithalamic H3K27-wildtype diffuse midline glioma, EGFR-altered with methylated MGMT. CASE: A 10-year-old female presented with an infiltrative bithalamic T2-hyperintense mass lacking diffusion restriction or contrast enhancement on MRI. Initial pathological inspection from biopsy was consistent with high-grade neuroepithelial tumor favoring high-grade glioma, however, immunohistochemistry was negative for H3K27M and demonstrated reduced H3K27-me3. DNA sequencing uncovered mutations in *EGFR* (exon 20 insertion) and *TP53* (R175H), with overexpression of *EGFR* and *CDK6* (but not *EZHIP*) identified by RNA-sequencing. Methylation profiling was consistent with high-grade glioma, matching closest with glioblastoma, IDH-wildtype, with positive MGMT promoter methylation. Treatment was initiated with focal chemoradiotherapy with concurrent temozolomide, with plans for adjuvant temozolomide/ lomustine.DISCUSSION: Our case adds to growing evidence suggesting bithalamic tumors represent a distinct genetic and epigenetic subset of diffuse midline gliomas often defined by H3K27-wildtype status, loss of H3K27-me3, and EGFR receptor alterations. Our patient's H3K27-wildtype, EGFR-altered tumor had reduced H3K27-me3 as well as positive MGMT promoter methylation, a molecular characteristic that has not been well-studied in H3K27-wildtype bithalamic gliomas, but is suspected to confer sensitivity to alkylating chemotherapy. The prevalence, prognostic impact, and therapeutic implications of MGMT promoter methylation in bithalamic H3K27-wildtype diffuse midline gliomas, including potential association with EGFR aberrations, requires further exploration.

HGG-32. DURABLE RESPONSE TO MTOR INHIBITOR AFTER FAILING CHECKPOINT INHIBITORS IN ULTRA-HYPERMUTATED HIGH GRADE GLIOMA IN CONTEXT OF CMMRD

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BACKGROUND: Paediatric High Grade Gliomas (HGG) have poor outcomes with conventional treatment. HGG in association with constitutional DNA mismatch repair deficiency (CMMRD) are hypermutated and have shown dramatic response to checkpoint inhibitors. Salvage following progression or failure to respond to check point inhibitors has rarely been reported. We describe a successful alternative therapeutic approach targeting the activated pathway (mTOR) in a hypermutated HGG. CASE SUMMARY: A 6-year-old girl presenting with seizures was diagnosed with left frontal lobe HGG with concurrent neck mass (Pilomatrixoma). Presence of synchronous tumours raised the

possibility of cancer predisposition; the HGG was hypermutated with germline PMS2 mutation confirming diagnosis of CMMRD. Near total resection was undertaken followed by focal radiotherapy 54 Gy, with 1 cycle of concomitant CCNU. MRI post radiotherapy showed tumour progression. Anti-PDl inhibitor Nivolumab was commenced. CTLA-4 antibody, Ipilimumab was added after 4 cycles of Nivolumab due to poor response. Tumour response was seen, but dual therapy had to be discontinued due to toxicity. The tumour progressed following further single agent Nivolumab. In view of multiple mutations in the mTOR pathway (NF1, PIK3/PTEN, TSC1, TSC2), a mTOR inhibitor, Everolimus was commenced. There was 25% tumour reduction after 4 weeks treatment and further reduction after 6 months. Resection of residual tumour showed necrotic tissue only. There continues to be a sustained response to Everolimus for over 12 months. **DISCUSSION:** Approximately a third of CMMRD HGG respond to checkpoint inhibitors. For those that don't, these hypermutated tumours offers the possibility of targeting specific molecular pathways. Response to Everolimus in HGG harbouring mTOR aberrations have been described. To our knowledge this is the first report of successful use of mTOR inhibitor in CMMRD HGG. **CONCLUSION:** Targeted molecular treatment for patients with CMMRD hypermutated brain tumours should be considered according to the mutated pathways.

HGG-33. PROGNOSTIC FACTORS OF H3K27M HISTONE-MUTANT DIFFUSE MIDLINE GLIOMAS IN PATIENTS ≤18YRS

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OBJECTIVE : Diffuse midline gliomas associated with high malignancy and poor prognosis. The primary treatment modalities include surgery, radiotherapy and chemotherapy. The purpose of this study was to investigate the prognostic factors of diffuse intracranial midline glioma. METHODS: A retrospective analysis was performed on 44 cases younger than 18 yrs of H3K27M histone-mutant diffuse midline gliomas diagnosed in Guangdong Sanjiu Brain Hospital from November 2017 to November 2021. The median age was 9 years (range:3-18), including 24 males and 20 females, lesions located in thalamus were 9, while in brain stem were 35. Treatment methods: 35 cases received radiotherapy, 9 cases did not. Among the patients who received RT, 26 cases with concurrent chemoradiotherapy + adjuvant chemotherapy, 8 cases with concurrent chemoradiotherapy only, and 1 case only with radiotherapy. Kaplan-meier method was used to calculate overall survival (OS), and log-rank test was used to test. $P < 0.05$ was considered statistically significant. RESULTS: By January 27, 2022, 13 cases survived, 25 cases died, and 6 cases were lost to follow-up. The median survival time of 44 patients was 6.95 months (range : 1-23.5 months). The median survival was 8.4 months in the radiotherapy group vs 3.7 months in the non-radiotherapy group ($P < 0.001$); The median survival time of radiotherapy without adjuvant chemotherapy vs radiotherapy with adjuvant chemotherapy was 6.2 months vs 9.35 months (P=0.479). CONCLUSION: Radiotherapy can prolong the survival time of diffuse midline glioma in children with H3K27M histone mutant but no survival benefit was observed in patients with concurrent chemotherapy.

HGG-34. UPFRONT MOLECULAR TARGETED THERAPY FOR THE TREATMENT OF BRAF-MUTANT PEDIATRIC HIGH-GRADE GLIOMA

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BACKGROUND: The prognosis for pediatric high-grade glioma (pHGG) is poor despite aggressive multi-modal therapy. Objective responses to targeted therapy with BRAF inhibitors have been reported in some patients with recurrent BRAF-mutant pHGG but are rarely sustained. METHODS: We performed a retrospective, multi-institutional review of patients with BRAF-mutant pHGG treated with off-label BRAF +/- MEK inhibitors as part of their initial therapy. RESULTS: Nineteen patients were identified, with a median age of 10.7 years (range: 1.8–20.3). Histologic diagnoses included HGG (n=6), glioblastoma (n=3), anaplastic ganglioglioma (n=4), diffuse midline glioma (n=3), high-grade neuroepithelial tumor (n=1), anaplastic astrocytoma (n=1), and anaplastic astroblastoma (n=1). Recurrent concomitant oncogenic alterations included CDKN2A/B loss, H3 K27M, as well as mutations in ATRX, EGFR and TERT. Eight patients received BRAF inhibitor monotherapy. Eleven patients received combination therapy with BRAF and MEK inhibitors. Most patients tolerated long-term treatment well with no grade 4–5 toxicities. Objective and durable imaging responses were seen in the majority of patients with measurable disease. At a median follow-up of 2.3 years (range,0.3–6.5), three-year progression-free (PFS) and overall survival (OS) for the cohort were 65% and 82%, respectively, and superior to a historical control cohort treated with conventional therapies. CONCLUSIONS: Upfront targeted therapy for patients with BRAF-mutant pHGG is feasible and effective, with superior clinical outcomes observed compared to historical data. This promising treatment paradigm is currently being evaluated prospectively in the Children's Oncology Group ACNS1723 clinical trial.

HGG-35. RADIATION INDUCED HIGH GRADE GLIOMAS: A SINGLE CENTER EXPERIENCE

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INTRODUCTION: Patients receiving cranial radiotherapy (RT) are at risk for a subsequent radiation-induced glioma (RIG). RIGs are rare, generally develop with a latency of 2 years to several decades, display high-grade histology and an aggressive clinical course with poor prognosis. METHODS: We retrospectively analyzed patients with a diagnosis of RIG seen at our institution from 2001-2021, analyzing clinical, histological, molecular, and genetic characteristics. RESULTS: Twenty-one patients (n=15 male) with a history of ALL (n=6), medulloblastoma (n=5), germ cell tumors (n=4), or other (n=6) diagnosed at a median age of 8.3 years (range 1.6 to 36.4) were identified. Median age at RIG diagnosis was 18 years (range 7.8 to 66.9). Prior RT was focal+craniospinal (n=7), whole brain (n=5), total body (n=3), focal (n=1), or unknown (n=5). Median radiation dose received was 2,340 cGy (range 1,200 to 5,400). The median time from RT to RIG diagnosis was 7.7 years (range 1.6 to 23.8). All RIGs were histologically high grade (WHO Grade III or IV). Immunohistochemistry did not reveal IDH(R132H) (n=9) or H3K27M (n=8) in any tumor. Some tumors demonstrated loss of expression of ATRX (1/9) and/or H3K27me3 (3/6), and/or strong diffuse expression of p53 (0/3). Targeted panel sequencing (n=10) revealed recurrent somatic alterations including CDKN2A/B, PDGFRa/KIT/KDR, TEK, MTAP, ATM and NF1. Germline alterations were detected in 4/12 patients (pathogenetic variants in ATM, CHEK2, HOXB13 and NF1). With median follow-up of 4.5 years, two-year PFS and OS for the cohort (n=20) were 10% and 44% respectively. Two patients (with anaplastic oligodendroglioma and anaplastic astrocytoma) are alive without progression 5.4 and 13.6 years after diagnosis following surgery, RT and chemotherapy. CONCLUSION: Although RIGs are associated with a poor prognosis, they are not always fatal. Our findings suggest aggressive therapy should be considered for these patients.

HGG-36. ELUCIDATING THE ROLE OF LONG NON-CODING RNAS IN PEDIATRIC HIGH GRADE GLIOMAS

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BACKGROUND: Genomic and transcriptomic studies have elucidated new insights into the landscape of diffuse intrinsic pontine glioma (DIPG). However, the role of long non-coding RNAs (lncRNAs) has not been explored at depth in these tumors, and there have not been studies focused on how lncRNAs interact with the K27M histone mutation. In a recent analysis of nearly 200 DIPGs and pediatric high-grade gliomas (pHGG), we previously detected a novel, recurring structural variant in the lncRNA CCDC26. This rearrangement occurs in nearly 10% of all DIPGs, and we