membrane potential and decreasing oxygen consumption rate along with extracellular acidification rate after abemaciclib/SpyADI monotherapy or its combination regimens. TEM confirmed damaged mitochondria and endoplasmic reticulum together with increased vacuolization under CDKi mono- and SEQ- CDKi/SpyADI combination therapy. SEQ-abemaciclib/SpyADI treatment suppressed the DSB repair system via NHEJ and HR, whereas SEQ-dinaciclib/SpyADI treatment increased γ-H2AX accumulation and induced Rad51/Ku80. The latter combination also activated the stress sensor GADD45 and β-catenin antagonist AXIN2. CONCLUSION: This study highlights the antitumoral potential of a combined SpyADI/CDKi approach. We show that sequential application of these substances has complex effects on mitochondrial dysfunction, invasiveness, and DNA-damage response. This provides a good starting point for further proof-of-concept studies to move forward with this strategy.

HGG-14. MOLECULAR CHARACTERIZATION OF UNIQUE BIOLOGICAL SUBGROUPS AMONG H3 WILD TYPE HIGH-GRADE GLIOMAS

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INTRODUCTION: Paediatric high-grade gliomas (HGG) are characterised by the aggressive biological behaviour with dismal prognosis of long-term survival 10-15%. Current molecular-biological diagnostic approaches allow for more precise characterization and determination of new unique subgroups of HGG. Our aim was to identify novel and rare HGG subgroups within our institution cohort. PATIENTS AND METHODS: Our reference centre patients´ cohort consisted of 97 clinically annotated patients with HGG diagnosed between 2000 and 2021. Sanger sequencing was used for screening of the most common HGG-related oncogenic drivers; furthermore we employed whole genome methylation array (Illumina Infinium MethylationEPIC BeadChip) and for selected samples RNA sequencing and expression profiling. RESULTS: Based on H3 status and previous radiotherapy we separated our HGG cases into the RIG, H3mut and H3wt groups. In contrast to H3mut(n=35) and RIG(n=11) that were uniformly fatal, H3wt group contained a proportion of long-term survivors. In the H3wt group we found patients carrying driver mutations in IDH1/2 (n=2) and BRAFV600E (7). Five young patients (under 3) consisted of 3 infant hemispheric gliomas (with NTRK and ROS1 fusions), one gliomatosis cerebri and one brainstem anaplastic astrocytoma with MYB/QKI fusion. We also identified a rare EWSR1-PATZ1 gene fusion in one patient. Importantly, long-term survivors recruited from these subgroups. On the contrary, four cases of MYCN GBM with poor prognosis presented in various locations: one disseminated, one gliomatosis cerebri and two with hemispheric tumour. We identified one patient with "hypermutated" glioblastoma and used targeted therapy with Nivolumab. In three samples of our patients with thalamic glioblastomas, we detected "loss of H3K27-trimethylation" caused by EZHIP overexpression. These tumours proved to be very aggressive with early metastatic recurrence and dismal prognosis. SUMMARY: Detailed characterization of H3 wild-type HGG is very important for further understanding of their biological behaviour, diagnostics, prognostication and identification of therapeutic targets.

HGG-15. GENERATION OF A NOVEL MOUSE MODEL FOR BRAIN TUMORS OF THE DNA METHYLATION CLASS "GBM MYCN"

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Multiple recent publications have described a highly aggressive subgroup of pediatric glioblastoma, which is clearly separable from other pediatric and adult glioblastoma based on its DNA methylation profile (GBM MYCN). These tumors almost exclusively occur in children and have a median overall survival of only 14 months. Many tumors in this group are driven by MYCN amplifications and harbor TP53 mutations. Otherwise, information about these tumors are still sparse and treatment is ineffective and causes severe side effects in many cases. In order to further investigate the biology and treatment options of these tumors, preclinical models are urgently needed. Here, we describe the generation of *hGFAP-cre::TP53Fl/Fl::lsl-MYCN* mice, which carry a loss of TP53 and show aberrant MYCN expression in neural precursors of the central nervous system. These animals develop large forebrain tumors within the first 80 days of life with 100 % penetrance. These tumors resemble human GBM MYCN tumors by histology, global gene expression, and DNA methylation. In order to understand the developmental biology and intratumoral heterogeneity, we employed single cell RNA sequencing (scRNAseq) to the murine tumors with first results indicating a resemblance of tumor cells to committed oligodendrocyte precursors. We further show that both murine and human tumor cells are sensitive to AURKA inhibition in vitro, suggesting a potential new therapeutic option for improved patient care. We believe that further characterization and utilization of the model will pave the way to improved treatment strategies for patients with these highly aggressive tumors.

HGG-16. FINAL ANALYSIS OF THE HIT-HGG-2007 TRIAL (ISRCTN19852453): SIGNIFICANT SURVIVAL BENEFIT FOR PONTINE AND NON-PONTINE PEDIATRIC HIGH-GRADE GLIOMAS IN COMPARISON TO PREVIOUS HIT-GBM-C/-D TRIALS.

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The aim of the HIT-HGG-2007 trial (ISRCTN19852453) was to demonstrate therapeutic non-inferiority of temozolomide radiochemotherapy for pediatric patients (3-18 years) with high-grade gliomas (pedHGG) in comparison to the cisplatinum-based radiochemotherapy of the two previous clinical trials HIT-GBM-C/-D. Between 06/2009 and 12/2016, 456 patients were enrolled at 79 centers in Germany, Austria, and Switzerland (n=18 dropouts, remaining

patients for confirmatory analysis: n=438). 438 patients from HIT-GBM-C/-D served as historical control. All pedHGG diagnoses had been confirmed by central neuroradiological and neuropathological review. Primary objective was achieved since non-inferiority of HIT-HGG-2007 in comparison to HIT-GBM-C/-D as indicated by 6 months event-free survival (EFS) was statistically confirmed (p=0.0125). Statistical survival analyses even revealed a better overall survival (OS) and EFS for HIT-HGG-2007 patients in comparison to their HIT-GBM-C/-D counterparts (EFS: p<0.0001; OS: p=0.0328). While EFS subgroup analyses for pontine and non-pontine pedHGG also showed a better survival of HIT-HGG-2007 patients (median EFS pontine pedHGG: 8.2 (n=152; confidence interval (CI): 7.6-9.4) versus 6.2 (n=170; CI: 5.5-6.9) months, p=0.0079; median EFS non-pontine pedHGG: 10.7 (n=276; CI: 9.6-12.4) versus 7.4 (n=267; CI: 6.4-9.2) months, p<0.0001), OS was only improved in HIT-HGG-2007 patients with non-pontine pedHGG (median OS non-pontine pedHGG: 19.3 (CI: 16.8-23.3) versus 16.2 (CI: 14.2-19.1) months; p=0.0181) but not with pontine pedHGG (median OS pontine pedHGG: 11.4 months versus 11.3 months, p=0.4021) Toxicity profile of HIT-HGG-2007 seemed very favorable with most CTCAE (common toxicity criteria adverse event) ≥ grade 3 as hematological toxicity, hepatotoxicity, and neurotoxicity. Less toxicity was observed during concomitant radiochemotherapy in comparison to HIT-GBM-C/-D. Further subgroup survival analyses as well as the assessment of the impact of MGMT promoter methylation are ongoing. In conclusion, our data show non-inferiority of the HIT-HGG-2007 trial with increased survival and less toxicity when compared with previous trials HIT-GBM-C/-D.

HGG-17. NOVEL FUSION IN CONGENITAL BRAINSTEM DIFFUSE HIGH-GRADE GLIOMA

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BACKGROUND: Infant-type hemispheric glioma, previously termed infantile glioblastoma multiforme, is a rare infantile neoplasm with improved survival and distinct molecular features when compared to other pediatric and adult-type high-grade glioma. Infant-type high-grade gliomas are typically located in the cerebral hemispheres and are characterized by ALK, ROS1, MET, and NTRK fusions. Typical brainstem gliomas (diffuse midline glioma, H3 K27-altered or diffuse intrinsic pontine glioma) are comparatively rare in this age group. As a result, the biology of brainstem congenital high-grade gliomas is poorly described. RESULTS: A 3 month old female who initially presented with failure to thrive had an apneic event and was found to have an infiltrative mass in the medulla with expansion into the pons and cervical spine on magnetic resonance imaging. She underwent surgical biopsy with pathology revealing diffuse high-grade glioma, WHO grade 4. Next generation sequencing showed no alterations to H3F3A, IDH, or fusions involving BRAF, ALK, ROS1, MET, or NTRK. Whole-transcriptome sequencing revealed a novel fusion of PDGFRB:APOBEC3C. She received chemotherapy with 2 cycles of carboplatin/etoposide and 2 cycles of carboplatin/etoposide/imatinib before having disease progression. She then underwent palliative radiation (35 Gy in 10 fractions) with near complete regression of her disease. Surprisingly, our patient has not had any progression of disease or new lesions now two years from her last therapy. CONCLUSION: Congenital high-grade glioma is a rare, unique entity that greatly differs from its adult and childhood counterparts. Here, we discuss a previously-unreported fusion of PDGFB:APOBEC3C in a patient with congenital brainstem diffuse high-grade glioma with a favorable clinical course. This highlights the importance of routine molecular characterization, both to better understand the complex biology of this rare disease and to guide prognosis and clinical decision making for individual patients and families.

HGG-18. LONG-TERM EFFICACY AND SAFETY OF LAROTRECTINIB IN PAEDIATRIC PATIENTS WITH TROPOMYOSIN RECEPTOR KINASE (TRK) FUSION-POSITIVE PRIMARY CENTRAL NERVOUS SYSTEM (CNS) TUMOURS

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INTRODUCTION: Neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions are oncogenic drivers in various tumours. Larotrectinib, a highly selective TRK inhibitor, demonstrated an objective response rate (ORR) of 75% across 206 evaluable patients with various non-primary CNS cancers (Hong et al, ASCO 2021). We report long-term data on larotrectinib-treated paediatric patients with TRK fusion-positive primary CNS tumours. METHODS: Patients aged <18 years with TRK fusion-positive primary CNS tumours enrolled in two clinical trials (NCT02637687, NCT02576431) were included. Larotrectinib was administered at 100 mg/m^2 (maximum: 100 mg) twice-daily. Response was investigator-assessed per RECIST v1.1 and RANO. RESULTS: As of July 2021, 28 patients with TRK fusion-positive primary CNS tumours were enrolled, including 14 high-grade and eight low-grade gliomas. Median age at enrolment was 7.0 years (range 1.0–17.0). Twenty-three patients (82%) received prior systemic therapy and 12 (43%) received prior radiotherapy. The ORR was 39% (95% confidence interval [CI] 22–59): three complete responses, eight partial responses, 15 stable disease and two progressive disease. The 24-week disease control rate was 82% (95% CI 63–94). Median duration of response (DoR) was not reached; median follow-up was 25.6 months. Median progression-free survival was 21.9 months (95% CI 9.2–not estimable). Median overall survival (OS) was not reached; median follow-up was 27.6 months. DoR and OS 24-month rates were 53% and 71%, respectively. Treatment duration ranged from 1.0 to 39.0+ months. Treatment-related adverse events (TRAEs) were mostly Grade 1–2. Grade 3–4 events occurred in three patients (increased gamma-glutamyltransferase, hyperglycaemia, hypernatraemia, hyponatraemia and neutropaenia). No patients discontinued treatment due to TRAEs. Fourteen patients progressed on treatment; four continued treatment post-progression for ≥4 weeks. CONCLUSION: Larotrectinib demonstrated high disease control rate, durable responses and a manageable safety profile. These results support testing for *NTRK* gene fusions in paediatric patients with primary CNS tumours.

HGG-19. CO-OCCURRENCES OF A HIGH-GRADE GLIOMA WITH CAVERNOUS MALFORMATIONS AND PATHOGENIC VARIANTS IN *PDCD10* AND *SMARCA4*

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INTRODUCTION: The co-occurrence of multiple disease processes can make for more challenging diagnoses. Here we report an unusual case of a patient found to have an *IDH1*-mutant high-grade glioma along with multiple cerebral cavernous malformations and pathogenic germline variants in *PDCD10* and *SMARCA4*. CASE DESCRIPTION: A 17-year-old female presented with left arm paresthesia and weakness along with persistent headaches within the frontal and occipital regions that progressed in intensity to include nausea and emesis. A fast sequence magnetic resonance imaging (MRI) of her head was obtained that revealed the presence of multiple bilateral cystic lesions suspicious for cavernomas, with the most notable lesion in the right parietal lobe. Ophthalmology consultation revealed grade III papilledema bilaterally. A full brain MRI with and without contrast was obtained and demonstrated a right anterior parietal lobe lesion with associated mass effect, as well as multiple bilateral supratentorial and left cerebellar cavernous malformations. The patient underwent tumor debulking of her dominant lesion. Pathology revealed an *IDH1*-mutant diffuse astrocytoma, WHO grade III. Tumor genetic testing was done and identified a *SMARCA4* and two *TP53* variants. Germline genetic testing was then pursued which revealed a *PDCD10* pathogenic variant consistent with familial cerebral cavernous malformation syndrome and a likely pathogenic variant in *SMARCA4*. Treatment of her high-grade-glioma included radiation therapy followed by maintenance oral temozolomide. DISCUSSION: This case illustrates the unusual co-occurrences of a high-grade glioma with familial cavernous malformation syndrome and germline pathogenic variants in PDCD10 and *SMARCA4*. Our patient continues to do well clinically, but because of her risk of developing small cell carcinoma of the ovary she has elected to undergo a prophylactic bilateral salpingo-oophorectomy. Recognition of abnormal genetic results is critical in the setting of multiple disease processes and can play a crucial role in the on-going care for a patient.

HGG-20. PRMT5 PROMOTES THE FORMATION AND GROWTH OF PEDIATRIC HIGH-GRADE GLIOMA BY MAINTAINING TUMOR STEM CELL POPULATIONS

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BACKGROUND: Pediatric high-grade gliomas (PHGG) are aggressive, undifferentiated CNS tumors comprising two broad subtypes: diffuse mid-