considered as IDH-/H3-wildtype gliomas (n=37/49, 75.5 %), mostly with a pedRTK2 subtype (n=15, 30.6%), followed by pedMYCN (n=5, 10.2 %). Within the IDH-/H3-wildtype gliomas, EGFR-altered tumors (n=10) seemed overrepresented. Survival analyses revealed a better OS for IDH1-mutant tumors (n=6; 54.6 vs. 15.2 months in IDH-wildtype; p=0.015) and a worse OS for TP53-mutant tumors (n=6; p=0.001). Despite the potential overrepresentation of EGFR-altered tumors, no other specific molecular markers for GC could be identified so far. Further analyses are ongoing. CONCLUSIONS: GC in children is confirmed as a poor prognostic phenotype include various epigenetic pediatric glioma subtypes, without a proven (epi)genetic mark of its own. The relevance of overrepresented EGFR alterations has to be determined yet.

HGG-50. SPECIFIC SENSITIVITY OF PEDIATRIC HIGH-GRADE GLIOMA WITH ATRX INACTIVATION TO PARP INHIBITOR COMBINATIONS

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Pediatric high-grade gliomas (pHGG) account for approximately 12% of pediatric brain tumors. Despite advances in molecular diagnosis and identification of discrete molecular subtypes, pHGG are the leading cause of cancer-related death in children. Thus, current research focuses on identifying novel therapeutic targets. Sequencing analyses across pediatric cancer types identified DNA repair perturbations as potentially targetable events in certain types of pediatric brain tumors. Herein, we investigated the potential of PARP inhibitors (PARPi), impeding the central role of PARP in DNA damage repair, in pHGG. We screened a patient-derived primary pHGG cell line panel (n=7) for their sensitivity towards 6 different PARPi (niraparib, olaparib, pamiparib, rucaparib, talazoparib, veliparib) using cell viability assays. Basal expression of DNA repair related proteins was assessed by immunoblot, and propidium iodide-based flow cytometry was used for cell cycle analysis. All pHGG were resistant towards single compound PARP inhibition. Interestingly, two *H3F3A-G34R* mutant pHGG models harboring inactivating *ATRX* mutations were characterized by elevated basal levels of pH2AX, suggesting increased stress resulting from DNA damage. Consequently, simultaneous targeting of PARP and other components of DNA repair in the respective models showed strong synergistic effects on cell viability, which was not observed to a comparable extent in other models such as *BRAFV600E/TERT* promotor mutant pHGG. Combination of talazoparib and irinotecan resulted in S-phase arrest. Within a precision oncology approach, we treated a 11-year-old child suffering from *H3F3A-G34R* mutant pHGG with *ATRX* mutation, that progressed during radiation, with niraparib and topotecan. The patient achieved partial remission and disease stabilization for 1 year. Taken together, PARPi combinations show potential for the treatment of pHGG with *ATRX* mutations. Currently, all cell models are characterized for DNA repair signatures by DNA sequencing. Further, in depth characterization of DNA damage responses upon concomitant PARP and topoisomerase inhibition in *ATRX*-mutated pHGG are ongoing.

HGG-51. UNCOVERING THERAPEUTIC VULNERABILITIES IN MISMATCH REPAIR-DEFICIENT GLIOMAS

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INTRODUCTION: We have observed that approximately 26% of recurrent gliomas acquire hypermutation following treatment with temozolomide (TMZ). Intriguingly, 91% of these tumors harbor mutations in mismatch repair (MMR) genes. Since MMR deficiency confers resistance to TMZ, strategies to target MMR-deficient gliomas stand to impact many patients. METHODS: We ablated the MMR genes *MSH2*, *MSH6*, *MLH1*, and *PMS2* using an all-in-one sgRNA-CRISPR/Cas9 expression vector to generate isogenic MMR knockouts in patient-derived glioma cell lines. We characterized the gene expression profiles of these MMR-deficient glioma models and leveraged high-throughput drug screens and genome-scale CRISPR/Cas9 dropout screens to identify therapeutic vulnerabilities induced by loss of MMR. RESULTS: We show that loss of each major MMR gene confers resistance to TMZ. Gene set enrichment analysis of our MMR-deficient knockouts shows enrichment of several hallmark gene sets including DNA repair and G2M checkpoint signatures, and our genome-wide CRISPR dropout screen reveals that MMR-deficient cells are preferentially dependent on a number of genes involved in DNA repair and cell cycle, along with several other pathways. Lastly, the high-throughput drug repurposing (REPO) screen shows that loss of MMR confers differential dependencies to small molecule inhibitors. CONCLUSIONS: Using CRISPR/Cas9 to knock out individual MMR pathway members allows us to systematically study the response of MMR-deficient cells to alkylating agents in an isogenic context. Importantly, these isogenic models reveal that MMR-deficient glioma cells possess novel genetic dependencies and sensitivities to small molecules, which may inform future therapies for MMR-deficient tumors.

HGG-52. COMBINATORIAL MODULATION OF HYPOXIC PATHWAYS LEADS TO ANTI-TUMORAL EFFECTS IN H3.3 K27M MIDLINE GLIOMAS

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Pediatric midline high-grade gliomas (pmHGG) are aggressive and incurable tumors of the central nervous system. There is a pressing need for novel therapeutic approaches to treat them. Therefore, proactive translational studies wish to go further discovering new targetable proteins and pathways. Our objectives are then to focus on the modulation of microenvironmental extrinsic features like intra-tumor hypoxia. To do so, we looked first on expressions of hypoxia biomarkers in a pool of patient-derived preclinical models of pmHGG and tested oxygen modulations, as well as hypoxia drug targeting. We designed subsequently our work in those models H3.3 mutated to evaluate balance between HIF1 and HIF2 expressions (immunofluorescence, RTqPCR, RNAseq and metabolomics) and to evidence the impact of hypoxia targeting combined to irradiation on cell proliferation, migration and metabolism. Hypoxia is inducing mainly HIF1 expression and its upstream and downstream pathways and is stabilizing HIF2 expression. Both HIFs are part of crucial survival signaling and represent targets to combine with irradiation. The use of their specific inhibitors shows an antiproliferative effect when HIF1 is downregulated. HIF2 inhibitors are stopping HIF2 transcriptional effect letting us uncover new pathways that this hypoxic inducible factor is regulating in pmHGG (stemness, glycolytic and aminoacid metabolism and histone expression). Together with irradiation this anti-hypoxic strategy seems to be highly effective on cell arrest and migration. Those results are confirming central roles of HIFs in pmHGG and their potencies in pmHGG therapies. The therapeutic efficiency is independent from p53 abnormalities in our models.

HGG-53. "PROFILE OF HIGH GRADE GLIOMAS AND DIFFUSE INTRINSIC PONTINE GLIOMAS IN GREEK PEDIATRIC PATIENTS: AN 8-YEAR SINGLE INSTITUTION'S EXPERIENCE"

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BACKGROUND/OBJECTIVES: Aggressive clinical and biological behavior, high morbidity and mortality are the main characteristics of pediatric high-grade-gliomas (HGG). Our aim was to study patients (pts) with HGG or diffuse-intrinsic-pontine-glioma (DIPG), diagnosed in the largest pediatric neurooncology Center in Greece. DESIGN-METHODS: We performed a retrospective-review of newly-diagnosed pts with HGG

or DIPG during 2014-2021. Gender, age, location, resectability, type of surgery, histological and molecular characteristics, management and outcome were analyzed. RESULTS: During the study-period, 38pts (18females), median age:9.35y(range:3days-16.9y), were diagnosed in our center. The most common tumor-location were pons (17/38 pts) and parietal lobe (11/38 pts). DIPG based on imaging-studies was diagnosed in 16pts. Surgical approach was performed in 32pts (VP-shunt insertion:8, biopsy:12, partial resection:6, subtotal: 6). In 23pts(5 brainstem-tumors) a histological-diagnosis was feasible. Astrocytoma grIV was found in 60.8% and grIII in 26.1%(14 and 6 respectively). Of notice, 3 additional pts with histological findings of low-grade(2grII and 1gr1), were upgraded in grIV after molecular-studies and DNA-methylation analysis. Furthermore, 17.3% of the pts (4/23, 3 located in the midline) carried a H3K27M-mutation (diffuse midline glioma, DMG), 17.3% a H3F3Amutation and 8.6% showed EGFR-overexpression. All patients>3years of age were treated upfront according to HIT-HGG2013 with radiotherapytemozolomide (29/32pts). In 5DIPG pts, reirradiation after diseaseprogression resulted in temporary symptomatic improvement. HGG-pts upon progression were treated with bevacizumab-irinotecan. Of the 38pts, 6pts elected to receive treatment in other countries. Overall-survival was 75.1%,15.1% and 3.7% at 1,2 and 3 years post-diagnosis respectively. Patients with DIPG/ DMG and non-midline HGG had a median overallsurvival of 1.10 years and1.34 years, respectively. CONCLUSIONS: The experience of our unit concurs with worldwide published series and shows that pediatric HGG and DIPG have a dismal prognosis. Re-irradiation may offer short survival prolongation. ASKNOWLEDGEMENTS: Authors asknowledge the contribution of KiTZ-Heidelberg in molecular diagnostics through collaboration with ACCC.

HGG-54. CLK1 ABERRANT SPLICING IN PEDIATRIC HIGH-GRADE GLIOMAS DISRUPTS KEY ONCOGENIC TRANSCRIPTIONAL PROGRAMS

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While much of the somatic coding variation underlying the oncogenic transformation of pediatric high-grade gliomas (HGGs) has been profiled, transcriptional splicing programs of these tumors remain under-explored. Here, we characterize aberrant alternative splicing in pediatric midline HGGs (n = 84). We identified 19,275 recurrent and significant (20% change from control, $P < 0.05$, FDR < 0.05) aberrant splicing events in 8,587 genes compared to non-diseased brainstem controls. Of those, 27% (n = 5,157) resulted in either a gain or loss of a known protein functional site within 3,294 genes. We prioritized splice variants affecting targetable kinases and found that mRNAs encoding CDC-like kinase 1 (CLK1), a known modulator of master splicing regulators, exhibit significantly increased exon 4 inclusion in midline HGGs. This leads to a gain of two known phosphorylation sites in CLK1, increased CLK1 protein expression and hyper-phosphorylation of Serine-rich splicing factors. To assess the impact of this event, we performed differential splicing and expression analyses, comparing tumors with the highest (n= 5) and lowest (n = 5) exon 4 inclusion. We discovered 3,037 genes to be differentially up-regulated in high exon 4 inclusion tumors with an enrichment of cancer-related pathways, including DNA repair, mitotic spindle, myogenesis and EMT. We next integrated these gene signatures with protein-protein interaction networks of kinase and transcription factors and show that increased CLK1 exon 4 inclusion disrupts critical regulatory networks, such as those involving FOXM1, which is implicated in cell cycle and proliferation processes. In summary, we describe aberrant splicing in pediatric HGGs as an additional mechanism that could drive tumorigenesis. Future work will focus on molecular validation and therapeutic targeting of CLK1 in available HGG models. Characterizing tumor-specific splicing variation has the potential to open new therapeutic strategies and understand mechanisms of treatment resistance in children with central nervous system tumors.

HGG-55. AN ADOLESCENT WITH A HIGH-GRADE GLIOMA: PERSUING A SPECIFIC DIAGNOSIS

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BACKGROUND: Recognizing the value of molecular parameters provides evidence with repercussions on diseases classification. CLINICAL CASE: A thirteen-year-old girl was admitted in coma at the Intensive Care Unit due to a hemorrhagic stroke. CT revealed an expansive left frontal lobe lesion with perilesional edema and compression signs by an extensive hematoma. She underwent an emergency decompressive craniotomy for hematoma drainage, and the lesion was biopsied. The subsequent MRI showed a large heterogeneous mass with a cystic component. A first subtotal resection of the lesion was performed considering the language area. The histopathological examination showed tumor cells organized in perivascular pseudorosettes around the central hyalinized thickened blood vessel throughout the tumor, a pattern typically encountered in astroblastoma. Immunochemistry stains revealed a positive reactivity for GFAP, Olig2, and synaptophysin. The molecular study found a BRAFV600E mutation and homozygous CDKN2A e CDKN2B deletions; no TERT amplification, BCORL1, nor MN1 gene rearrangements were identified. She completed six weeks of radiotherapy without neurological signs/symptoms. A new surgical intervention was done 12 weeks later due to a slight increase of residual lesion. The rapid growth and the presence of viable tumor cells justified the therapy with irinotecan and bevacizumab, with mild adverse effects. One year after diagnosis, the adolescent is clinically well, without neurologic deficits and the MRI without evidence of residue or tumor recurrence. DISCUSSION: We began therapy based on morphological diagnosis. Complete resection and radiotherapy proved to be beneficial as in other high-grade gliomas, but the value of other therapies is still unknown. The BRAF gene variant would allow treatment with dabrafenib (in association with trametinib) or vemurafenib; however, she presents molecular findings associated with poor prognosis (CDKN2A deletion) that may impair the effectiveness of these therapies. According to the new WHO classification, the absence of MN1 alterations precludes the diagnosis of astroblastoma.

HGG-56. SPATIAL MAPPING OF THE TUMOR MICRO-ENVIRONMENT IN PEDIATRIC GLIOMA

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High-grade glioma are the main cause of cancer-related death in children. The highly heterogeneous composition of the tumor cells and their interactions with the tumor micro-environment (TME), contribute substantially to the poor response to treatment and the high levels of morbidity and mortality. Here, we used high-dimensional, multiplexed immunohistochemistry to map the single-cell tissue architecture of 26 pediatric glioma samples covering 8 histologic diagnoses, allowing us to determine the spatial distribution of the various tumoral subtypes and how these interact with their local immune-microenvironment. Overall, this analysis showed that tumor grade anti-correlated with the amount of infiltrating cytotoxic T-lymphocytes (CTLs), which were typically more exhausted in the higher grade tumors. In addition, tumor associated macrophages were primarily infiltrating from the blood and presented an M2-like anti-inflammatory phenotype which became more extended with tumor grade. Using the spatial information, possible cell-cell interactions could be determined. In lower grade glioma, we observed an increased activation level of CTLs that were closely located to neighboring T-helper cells. In pediatric glioblastoma, on the other hand, CTLs, even though they were located close to a T-helper cell, could only minimally be activated, and showed more extended exhaustion when residing further away. Additionally, the activation of the CTLs was associated to the distance to the closest PD-L1 positive macrophage in pilocytic astrocytoma and desmoplastic infantile ganglioglioma. In conclusion, with the use of multiplex immunohistochemistry, we are able to study the tumor and TME of pediatric glioma in depth on a single-cell and spatial level, which allows us to further study the heterogeneous landscape of these tumors.

HGG-57. BARRIERS IN THE MANAGEMENT OF PEDIATRIC HIGH-GRADE GLIOMAS IN A LOW-RESOURCE SETTING DURING THE COVID ERA

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INTRODUCTION: High-grade gliomas account for <5% of all pediatric brain tumors with a 20% 5-year overall survival even with maximal safe resection followed by concurrent radiotherapy and chemotherapy. Patients