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