line glioma with H3K27M mutations (DMG) and cortical high-grade glioma (H3K27-wild-type (wt) PHGG). During normal development, PRMT5 promotes stem cell self-renewal through methylation of arginine residues in histone tails. We hypothesized that PRMT5 controls self-renewal essential to the proliferation of PHGG tumor initiating cells (TICs). METHODS: We identified PRMT5 as potentially oncogenic in PHGG through a screen of 4,139 shRNAs targeting 406 genes with epigenetic activity. To elucidate PRMT5's activity, we used lentiviral shRNA delivery to knock down (KD) PRMT5 expression in four DMG and one H3K27-wt PHGG cell lines. We performed in vitro growth, cell cycle, apoptosis, limiting dilution and bulk RNA-Seq assays to determine the phenotypic effects of PRMT5 KD. To identify PRMT5's gene targets, we performed cleavage under targets & release using nuclease (CUT&RUN) followed by qPCR and are currently performing CUT&RUN-Seq. We orthotopically implanted PRMT5 KD PHGG cells into mice and tracked survival, tumor growth and tumor histological characteristics. RESULTS: In vitro, PRMT5 KD reduced cell growth ($p<0.001$), slowed cell cycle progression and increased apoptosis. PRMT KD also slowed neurosphere formation, demonstrating reduced self-renewal ($p<7E-9$). Geneset expression analysis showed PRMT5 KD reduced expression of self-renewal genes and increased expression of differentiation genes (FDR<0.0001). In vivo, PRMT5 KD reduced tumor growth, as monitored by bioluminescence and MRI, and aggressiveness, based on Ki-67 staining ($p<0.05$), leading to increased survival ($p<0.001$). CUT&RUN-qPCR results showed PRMT5 KD led to decreased expression and H3K4me3 promoter occupancy at PAX3, and decreased expression and increased H3K27me3 occupancy at S100A6. PAX3 and S100A6 are oncogenes that preserve TIC self-renewal. CONCLUSION. In vitro experiments show that PRMT5 KD epigenetically reduces TIC self-renewal. In vitro and in vivo, PRMT5 KD reduced PHGG tumor cell growth and aggressiveness.

HGG-21. ONCOGENIC TYROSINE KINASE GENE FUSIONS IN INFANT-TYPE HEMISPHERIC GLIOMAS - COMPARISON OF RNA- AND DNA-BASED METHODS FOR THEIR RELIABLE DETECTION

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High-grade diffuse gliomas (HGG) in early childhood are characterized by a more favorable outcome compared to older children. We demonstrated in previous studies that these tumors have stable genomes. Activating tyrosine kinase gene fusions in infant-type hemispheric gliomas represent therapeutic targets. 50 supratentorial HGG occurring in children younger than four years were retrieved from the archives of the Brain Tumor Reference Center, Institute of Neuropathology, Bonn University. DNA and RNA were extracted from FFPE tumor samples. Gene fusions were identified on the DNA level by FISH using break-apart probes for *ALK, NTRK1, -2, -3, ROS1* and *MET* and Molecular Inversion Probe (MIP) methodology. On the RNA level, fusion transcripts were detected by targeted RNA sequencing as well as Nanostring assay with fusion-specific probes. 37 supratentorial HGG occurred in the first year of life, 13 HGG between one and four years. 18 cases showed fusions of *ALK* to different partners; all occurred in the first year of life (18/37, 48.6%). Fusions of *ROS1* were found in 5, *MET* in 3, *NTRK1, -2, -3* in 10 cases. 12 cases showed no and two cases novel fusions. The different methods led to comparable results. Only recurrent fusions with known fusion partners were detectable with fusion sequence-specific Nanostring probes and library construction for targeted RNA sequencing failed in a fraction of cases. Break-apart FISH led to reliable results on the next day, and MIP technology represented the most sensitive method for analysis of FFPE samples. Gene fusions involving the tyrosine kinase genes *ALK, MET, ROS1* and *NTRK1, -2, -3* occurred in 72% of HGG of young children; most frequent were ALK fusions occurring in tumors of infants. DNA-based MIP technology represented the most robust and sensitive assay. A combination of RNA- and DNA-based methods to detect these fusions with high reliability is recommended.

HGG-22. UPTAKE OF INVESTIGATIONAL THERAPY IN CHILDREN WITH HIGH GRADE GLIOMA

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High grade gliomas (HGG) in children carry a dismal prognosis. Standard therapy includes resection when possible, radiotherapy and sometimes the addition of temozolomide. There is no standard treatment for progression or relapse. Since November 2018 we have offered upfront molecular testing to all children with HGG who had biopsy/ resection. Testing was mainly done by next generation sequencing panel, and some had research based methylation profiling and RNA seq. We aimed to see whether families chose to receive additional investigational treatment as a result of the molecular testing results and whether the treatment involved participation in a clinical study, or whether treatment was compassionate. A total of 22 patients aged 2.8-16.8 years with HGG had a biopsy/resection over this three year period. Thirteen had diffuse midline glioma (DMG) of which 11 had the H3K27 mutation, and 9 were cortical. Six children had underlying predisposition syndromes: mismatch repair deficiency (n=3 proven + 1 highly suspected), neurofibromatosis1 (n=1), Li-Fraumeni (n=1). All the cortical gliomas had potential treatment options based on their molecular testing. 10/22 (45%) children received investigational therapy of which only three participated in a clinical study while the rest received compassionate therapy. Compassionate treatments included BRAF/MEK inhibitors (n=4), Larotrectinib (n=1), and immune checkpoint inhibitors (n=2). Of the 12 who did not receive investigational therapy, four, all cortical, have potential therapy options but are currently in remission. Of the remaining eight, two had very rapid clinical deterioration and died, and six (all DMG) did not wish/ were unable to travel abroad and no relevant clinical study was available locally. We conclude that the families of children with HGG are highly motivated to receive investigational therapy. Upfront molecular testing of these tumors, especially for cortical HGG, is imperative and there is a growing need for accessible clinical studies.

HGG-23. THE PRESENCE OF *ROS1* FUSIONS ARE NOT LIMITED ONLY TO INFANTILE HEMISPHERIC GLIOMA.

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INTRODUCTION: Diffuse pediatric-type high-grade gliomas are diffuse gliomas with histological features of malignancy, typically occurring in children, and infants. For these tumors, precise classification, identification of prognostic and predictive factors requires molecular analysis. The ROS proto-oncogene 1 (*ROS1*) gene encodes a receptor tyrosine kinase that is involved in chromosomal rearrangements in numerous malignancies, and may be an attractive therapeutic target, since specific inhibitors have been approved for several neoplasms. Molecular evaluation including detection of *ROS1* fusions in pediatric gliomas are not included in standard diagnostic tests so far, therefore data on its significance is still limited. We present two cases of pediatric *ROS1* fusion-positive brain tumors. METHODS AND RESULTS: The patient no.1 was 1 year old boy with disseminated brain lesions. Histopathological examination displayed the presence of a neoplasm, which was composed of round and spindle-shaped cells with palisading necrosis, mitotic activity, and microvascular proliferation. The patient no.2 is 9 years old girl with tumor located in left frontal lobe. Microscopically, the neoplasm revealed the presence of oligodenroglial-like component with microvascular proliferation, and high mitotic activity. Targeted gene sequencing panel - Ampliseq Childhood Cancer Panel for Illumina was used to detect diagnostic and targetable gene fusions. In both of patients *ROS1:GOPC* gene fusions were detect. Identified fusions allowed to established diagnosis - infant-type hemispheric glioma with *ROS1* fusion (patient no 1) and - diffuse pediatric-type high grade glioma with *ROS1* fusion (patient no 2). CONCLUSIONS: Our results indicate, that the presence of *ROS1* fusions are not limited to the infant-type hemispheric gliomas only and may play a role in other glioma entities. It may be worth to include this biomarker in the diagnostic panel of pediatric brain tumors to establish a more precise diagnosis and a potential therapeutic target. Funded by National Science Centre, Poland (2016/23/B/NZ2/03064).

HGG-24. NTRK-REARRANGED INFANTILE GLIOMAS OF SUPRASELLAR/ OPTIC PATHWAY ORIGIN

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BACKGROUND: Oncogenic fusions involving neurotrophic tyrosine receptor kinase (NTRK) have been identified across cancer types and represent potential therapeutic targets given availability of TRK inhibitors. Gliomas harboring NTRK fusions have been described most commonly in infants with high-grade histology and hemispheric tumor location, though there is emerging evidence suggesting clinical, histopathologic, and molecular heterogeneity. Herein, we present two cases of NTRK-rearranged suprasellar/ optic pathway gliomas. CASE DESCRIPTIONS: The first patient was diagnosed at 6 months old with an extensive suprasellar/ optic pathway tumor, treated with carboplatin/ vincristine, which initially re-