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Replication repair deficiency (RRD) is an important driving mechanism of pediatric high grade glioma (pHGG) occurring predominantly in the context of germline mutations in RRD-associated genes. Although pHGG present specific patterns of DNA methylation corresponding to driving oncogenic processes, methylation patterns have not been well studied in RRD tumors. We analyzed 52 RRD pHGG using either 450k or 850k methylation arrays. These arrays were compared with 234 PHGG driven by other genetic or epigenetic mechanisms and 10 additional pHGG samples known to be hypermutant. RRD pHGG displayed a methylation pattern corresponding to specific secondary mutations such as IDH1 and H3K27M. Strikingly, RRD pHGG lacking these known secondary mutations largely clustered together with a poorly described group previously labelled Wild type-C. Most of the hypermutant tumors clustered in a similar location suggesting undiagnosed RRD may be a driving force for tumors clustering in this location. Analysis of methylation patterns revealed that RRD pHGG displayed a unique CpG Island Demethylator Phenotype in contrast to the Methylator Phenotype described in other cancers. This effect was most concentrated at gene promotors. Prominent demethylation was observed in genes and pathways critical to cellular survival including cell cycle, gene expression, cellular metabolism and cellular organization. These data suggest that methylation profiles may provide diagnostic information for the detection of RRD pHGG. Furthermore, our findings highlight the unique natural selection pressures in these highly dysregulated, hypermutant cancers and provide novel impact of hypermutation and RRD on the cancer epigenome.

## HGG-21. GERMLINE MUTATIONS IN MSH2 GENE IN PEDIATRIC PATIENTS WITH CONGENITAL AND SPORADIC GLIOBLASTOMA Maria Ejmont, Małgorzata Rydzanicz, Wiesława Grajkowska, Marta Perek-Polnik, Agnieszka Sowińska, Magdalena Kozłowska, Maria Łastowska, Maciej Pronicki, Rafał Płoski, Bożenna Dembowska-Bagińska, and Joanna Trubicka; The Children's Memorial Health Institute, Warsaw, Poland

INTRODUCTION: Glioblastoma (GBM) remains one of the biggest therapeutic challenges in neuro-oncology. In spite of multimodal treatment approaches the prognosis of GBM is extremely poor, median survival is estimated about 12-16 months. Although GBM is one of the most common and malignant primary brain tumors, pediatric glioblastoma, including congenital is a very rare tumor, with an incidence of about 1.1-3.4 per million live births. Moreover, the mode of presentation, behavior, response to therapy and molecular background of pediatric glioblastomas differs from adult type of GBM. Until now, about ten patients with congenital glioblastoma have been described and in none of them germline markers were examined. Here we report two patients with GBM, one with congenital tumor with germline mutations in MSH2 gene. METHODS: Targeted Next-Generation Sequencing (NGS) of the probands DNA extracted from leucocytes was performed using the TruSight One sequencing panel on an Illumina HiSeq 1500. Applied gene panel investigated the coding sequence and splice sites of 4813 genes associated with known disease phenotypes. The NGS data were analyzed using an in-house procedure. Identified variants were validated by Sanger sequencing. RESULTS: NGS analysis of patients constitutional DNA revealed know, pathogenic variants c.940C>T and c.942 + 3A>T in MSH2 gene (NM\_000251.3) associated with MMR-dependent hereditary cancer syndromes. CONCLUSION: Molecular analysis are heavily needed for better understanding of pediatric GBM etiology and new treatment modality implementation. Identification of this oncogenic driver may provide insight into the pathogenesis of GBM, including congenital cases. Funded by National Science Centre, Poland (2016/23/B/NZ2/03064 and 2016/21/B/ NZ2/01785).

## HGG-24. HIGH-GRADE GLIOMA WITH A NOVEL FUSION GENE OF VCL-ALK

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A previously healthy 2-year-old boy presented with status epilepticus following intermittent vomiting. Computed tomography scan showed a 7cm mass on the left occipital lobe with midline shift, inferior cerebellar herniation, and diffuse cerebral edema. The extensive dissemination to bilateral cerebral hemispheres, brain stem, and optic nerve was also observed. He underwent brain biopsy from the lesion on his left occipital lobe. The histopathological diagnosis determined the diffuse or epithelial proliferation of astrocytic tumor cells with high mitotic rate, positive for p53 and glial fibrillary acidic protein positive staining consistent with high-grade glioma. The progressive tumor led to communicating hydrocephalus, that was favorably controlled by cerebrospinal fluid shunting. The data from the FoundationOne CDx cancer genome profile disclosed a novel VCLanaplastic lymphoma kinase (ALK) fusion in the tumor cells of the patient. ALK rearrangement was determined to be positive for the tumor cells assessed by fluorescence in situ hybridization. Ônly 4 pediatric cases of glioma with ALK-rearrangement have ever been reported. All of them received subtotal or gross total resections and then survived with or without chemotherapy. This is the first case of glioma harboring VCL as a novel partner of ALK fusion gene. After the favorable response to the first-line chemotherapy, subsequent irradiation therapy has now been scheduled. The molecular classification of high-grade glioma may help to expand the targeted therapy for unresectable advanced brain tumor.

## HGG-26. H3G34V MUTATION AFFECTS GENOMIC H3K36 METHYLATION IN PEDIATRIC GLIOMA

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BACKGROUND: Histone H3.3 mutation (H3F3A) occurs in 50% of cortical pediatric high-grade gliomas. This mutation replaces glycine 34 with arginine or valine (G34R/V), impairing SETD2 activity (H3K36-specific trimethyltransferase), resulting in reduced H3K36me on H3G34V nucleosomes relative to wild-type. This contributes to genomic instability and drives distinct gene expressions associated with tumorigenesis. However, it is not known if this differential H3K36me3 enrichment is due to H3G34V mutant protein alone. Therefore, we set to elucidate the effect of H3G34V on genomic H3K36me3 enrichment in vitro. METHODS: Doxycyclineinducible short hairpin RNA (shRNA) against H3F3A was delivered via lentivirus to established H3G34V mutant pediatric glioma cell line KNS42, and H3G34V introduced into H3.3 wild type normal human astrocytes (NHA). Transfections were confirmed by western blot, fluorescent imaging, and flow cytometry, with resulting H3.3WT and H3K36me3 expression determined by western blot. H3.3WT, H3K36me3, and H3G34V ChIP-Seq was performed to evaluate genomic enrichment. RESULTS: Complete knockdown of H3G34V was achieved with DOX-induced shRNA, with no change in total H3.3, suggesting disproportionate allelic frequency of genes encoding H3.3 (H3F3A and H3F3B). Modest increase in H3K36me3 occurred after H3F3A-knockdown from KNS42, suggesting H3G34V alone impacts observed H3K36me3 levels. Distinct H3K36me3 genomic enrichment was observed with H3G34V knock-in. CONCLUSIONS: We demonstrate that DOX-inducible knockdown of H3F3A in an H3G34V mutant pediatric glioma cells and H3G34V mutation transduction in wild-type astrocytes affects H3K36me3 expression. Further evaluation by ChIP-Seq analysis for restoration of wild-type genomic H3K36me3 enrichment patterns with H3G34V knockdown, and mutant H3K36me3 patterns with H3G34V transduction, is currently underway.

## HGG-27. ANTI-CANCER POTENTIAL OF ARGINASE FOR HIGH-GRADE GLIOMA IN VITRO & IN-VIVO

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BACKGROUND: High-grade glioma is currently incurable. It was reported that glioma may be auxotrophic to arginine due to the lack of urea cycle genes expressions, suggesting arginase may be a potential agent for high grade glioma. AIM: We investigated the efficacy of pegylated arginase I (pegArg-I) or in combination with other anti-cancer drugs for high-grade glioma *in vitro* and *in vivo*. METHODS: 4 high-grade glioma cell lines (U87, U373, U138, D54) were treated with pegArg-I *in vitro*. The molecular mechanism of pegArg-I-induced cytotoxicity was tested in U87. The ultra-morphological changes of pegArg-I-treated U87 was investigated by both scanning and transmission electron microscopy. Orthotopic glioma xeno-graft model with luciferase-transfected U87 cell line was tested for anticancer efficacy of peg-Arg I *in vivo*. RESULTS: We showed that pegArg-I