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BACKGROUND: Data about high-grade glioma (HGG) in very young children (≤3 years old at diagnosis) is scarce. METHODS: 180 pediatric HGG patients were treated at the Children Cancer Hospital - Egypt (CCHE-57357) between July 2007 and June 2018, with 17 patients aged ≤3 years at diagnosis. Medical records were retrospectively reviewed for clinical, radiological and histopathological data, treatment received and survival outcome. RESULTS: Median age was 29.2 months (range: 2.4 – 35.8 months; males = 9). Most frequent pathological diagnosis was Glioblastoma, WHO grade-IV (n = 11, 64.7%) and one patient had H3-mutant diffuse midline glioma. All patients underwent surgery (gross-total resection, n = 6, 35.3%; subtotal-resection, n = 5, 29.4%; biopsy, n = 6, 35.3%). One patient (age = 7 months) progressed and died before starting adjuvant therapy. All patients ≤1 year of age (n = 5) received adjuvant chemotherapy (CT) only, older children (n = 11) received adjuvant radiotherapy (RT) (total dose range: 54 – 60 Gy) and CT (CCG-945 protocol). The 1-year overall survival (OS) rate was 47.1%; and event-free survival (EFS) rate was 35.3%. EFS differed between those who received RT and those who did not (1-year EFS 54.5% and 0% respectively, p = 0.001). Compared to older children, anatomical distribution of tumors was significantly different with non-midline locations being the commonest in patients ≤3 years old (88.2% vs 46.4%, p=0.01). CONCLUSIONS: HGG in very young children arise predominantly in non-midline locations and usually lack the H3-mutation. RT seems crucial in the management of pHGG regardless of age subgroup.

HGG-18. CLINICAL EFFICACY OF ONC201 IN THALAMIC H3 K27M-MUTANT GLIOMA

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ONC201, a bitopic DRD2 antagonist and allosteric ClpP agonist, has shown encouraging efficacy in H3 K27M-mutant glioma. Given that the thalamus has the highest extra-striatal expression of DRD2, we performed an integrated preclinical and clinical analysis of ONC201 in thalamic H3 K27M-mutant glioma. ONC201 was effective in mouse intra-uterine electroporation (IUE)-generated H3 K27M-mutant gliomas, with an *in vitro* IC_{50} of 500 nM and 50% prolongation of median survival *in vivo* (p=0.02, n=14). We analyzed thalamic H3 K27M-mutant glioma patients treated with ONC201 on active clinical trials as of 5/22/19 enrollment (n=19 recurrent and 10 post-radiation, non-recurrent; 5–70 years old). As of 12/18/2019, PFS6 and OS12 are 26.3% and 36.8%, respectively, in the recurrent group. For non-recurrent patients, with median follow up of 21.9 months (8.6– 26.6) from diagnosis, median PFS or OS have not been reached. This surpasses historical OS of 13.5 months. Best response by RANO includes 1 CR, 3 PR, 4 SD, 8 PD for recurrent patients and 2 PR, 4 SD, 1 PD for nonrecurrent patients (4 on-trial patients experienced regressions that are yet unconfirmed responses). Median duration of response for recurrent patients is 14.0 months (2.0–33.1). Furthermore, H3 K27M cell-free tumor DNA in plasma and CSF correlated with MRI response. In summary, single agent ONC201 administered at recurrence, or adjuvantly following radiation, demonstrates promising clinical efficacy in thalamic H3 K27M-mutant glioma patients who currently have no effective treatments following radiation. Investigations are ongoing to assess whether micro-environmental DRD2 expression explains the early exceptional responses in thalamic H3 K27M-mutant glioma.

HGG-19. IDENTIFICATION OF NOVEL SUBGROUP-SPECIFIC MIRNA EXOSOMAL BIOMARKERS IN PEDIATRIC HIGH-GRADE GLIOMAS

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Pediatric high-grade gliomas (pHGG) are heterogeneous brain tumors for which new specific diagnostic/prognostic biomarkers are needed. In this study, we aimed to identify new pHGG subgroup specific biomarkers by exploiting exosomes, known vehicles of oncogenic signals. We used plasma from 23 patients (including 6 controls) and conditioned medium from 12 patient-derived cell-lines, representing all locational and molecular subgroups. Upon exosome isolation, total RNA was extracted and miRNAs were assessed using a PCR Panel. Analysis of plasma miRNome showed that tumor exosomal samples were largely clustered together, independently from their locational and/or molecular subgroup. We identified 20 significantly upregulated and 25 downregulated miRNAs compared to controls. Interestingly, 27 miRNAs were expressed only in tumors. Furthermore, the unsupervised clustering showed a clear separation based on locational (hemispheric *vs* pontine) and mutational (WT *vs* H3.3G34R or H3.3G34R *vs* H3K27M) subgroup comparisons, with the identification of distinct miRNomes underlying the key role of location and mutations in defining the pHGG exosomal miRNA profile. This was further confirmed analyzing the miRNOme from cell-line derived exosomes. Moreover, we identified a pool of significantly differentially regulated miRNAs in diagnose *vs* relapse and biopsy *vs* autopsy cell-lines. Most importantly, when comparing hemispheric *vs* pontine and H3.3G34R *vs* H3.3K27M, we identified respectively four and three miRNAs equally dysregulated and in common between plasma and cell-lines. Those were strongly associated mainly to transcriptional regulation and targeting *TTC9*, linked to cancer invasion and metastasis. Based on this, we suggest exosomal miRNAs as a powerful new pHGG diagnostic/prognostic tool.

HGG-20. DIAGNOSTIC AND BIOLOGICAL ROLE OF METHYLATION PATTERNS IN REPLICATION REPAIR DEFICIENT HIGH GRADE GLIOMAS

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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

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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 7 cm 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 VCL-anaplastic 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. Only 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: Doxycycline-inducible 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 xenograft model with luciferase-transfected U87 cell line was tested for anti-cancer efficacy of peg-Arg I *in vivo*. RESULTS: We showed that pegArg-I