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INTRODUCTION: Diffuse midline gliomas have unfavorable prognoses due to the difficulty of surgery and chemo-radiation resistances. The purpose of this study is to overview our surgical experiences and prognoses of this challenging neoplasm. MATERIALS AND METHODS: Five patients of diffuse midline gliomas who were treated between 2016 and 2019 were enrolled. Tumor locations, surgical procedures, molecular diagnoses, and prognoses were retrospectively reviewed. RESULTS: There were 3 male and 2 female patients, and the median age was 15 years ranged from 7 to 21 years. Tumors were located at the basal ganglia in 1 patient, thalamus in 1, brain stem in 2, and cervical spine in 1. Mutations of *H3 K27M* genes were detected in 4 surgically treated patients, except for 1 patient, who were radiologically diagnosed as diffuse intrinsic pontine glioma (DIPG). Focal irradiation of ranged 35 to 54Gy were administered in all cases along with temozolomide in 2 cases and bevacizumab in 2 cases. The median survival time was 13 months ranged from 4 to 18 months. DISCUSSION: Supratentorial tumors were maximumly resected, whereas just biopsies were performed in cases of exophytic brain stem and spinal tumors. Diagnosis of DIPG was made without using surgical specimens. Therapeutic strategies should be discussed with a concern to the patients' qualities of life for this tumor entity with dismal prognosis.

DIPG-05. HISTONE H3.3 K27M IMPAIRS SER31 PHOSPHORYLATION, RESULTING IN CHROMOSOMAL INSTABILITY, LOSS OF CELL CYCLE CHECKPOINT CONTROL, AND TUMOR FORMATION

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Diffuse midline gliomas with the H3.3 K27M mutation are lethal brain tumors in children. H3 K27M causes global loss of Lys27 triple methylation (Lys27me3), inducing epigenetic reprograming. Here we show that H3.3 K27M also causes decreased H3.3 Ser31 phosphorylation on mitotic chromosomes. We show that H3.3 K27M DIPG cells have reduced pericentromeric phospho-Ser31 and increased rates of chromosome missegregation compared to normal, diploid human cells. CRISPR-editing K27M to M27K restored phospho-Ser31 to WT levels and dramatically decreased the rate of chromosome missegregation. We confirm that Chk1 is the H3.3 Ser31 kinase: K27M mutant H3.3 protein exhibits ~60% reduced Chk1 phosphorylation of Ser31 in vitro. Chk1 knockdown completely abolishes phospho-Ser31 in cells and these have increased rates of chromosome missegregation. In normal, diploid cells, expression of K27M or an S31A non-phosphorylatable mutant increased chromosome missegregation; this is suppressed by expressing a phosphomimetic double mutant (K27M/S31E) that restores phospho-Ser31. WT cells arrest following chromosome missegregation. However, cells expressing H3.3 K27M or S31A fail to arrest - despite having WT p53. Finally, we expressed H3F3AS31A and PDGFb in an RCAS/TVA mouse model of DIPG and ~80% developed diffuse high-grade brain tumors and show significantly decreased survival. Our results suggest that loss of phospho-Ser31 alone is oncogenic because H3.3 S31A-expressing cells are WT for K27me3. Our results demonstrate that H3.3 K27M inhibits Ser31 phosphorylation both in vitro and in vivo, leading to both chromosome missegregation and loss of subsequent G1 arrest – thus creating diffuse midline gliomas with both dynamic, complex karyotypes and epigenetic reprogramming.

DIPG-07. HIGH THROUGHPUT DRUG SCREENING IDENTIFIES POTENTIAL NEW THERAPIES FOR DIFFUSE INTRINSIC PONTINE GLIOMAS (DIPGS)

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DIPGs are the most devastating of all brain tumors. There are no effective treatments, hence almost all children will die of their tumor within 12 months. There is an urgent need for novel effective therapies for this aggressive tumor. We performed a high-throughput drug screen with over 3,500 biologically active, clinically approved compounds against a panel of neurosphere-forming DIPG cells. We identified 7 compounds- auranofin, fenretinide, ivermectin, lanatoside, parthenolide, SAHA and mefloquine- that were confirmed to have potent anti-tumor activity against a panel of DIPG-neurospheres, with minimal effect on normal cells. Using cytotoxicity and clonogenic assays, we found that these drugs were able to inhibit DIPG-neurosphere proliferation and colony formation *in-vitro*. To determine whether the *in-vitro* efficacy could be replicated *in-vivo*, we tested the activity of each of these compounds in an orthotopic DIPG model. Of the agents tested, fenretinide and SAHA were the most active anti-tumor agents, significantly enhancing the survival of tumor bearing animals. Mechanistic studies showed fenretinide enhancing apoptotic cell death of DIPG cells via inhibition of PDGFRa transcription and downregulation of the PI3K/AKT/MTOR pathway. We therefore examined the therapeutic efficacy of fenretinide using a second orthotopic model with PDGFRa amplification. We used two different Fenretinide formulations (LYM-X-Sorb and NanoMicelle) which were found to enhance survival. Fenretinide is clinically available with safety data in children. Validation of the activity of Fenretinide in PDGFRa-amplified or overexpressed DIPGs will lead to the development of a clinical trial, allowing the advancement of fenretinide as potentially the first active therapy for DIPG.

DIPG-08. ELECTRONIC SEQUENCING PROVIDES OPTIMIZED QUANTIFICATION OF SERIAL, MULTI-GENE MOLECULAR RESPONSE IN THE CSF OF CHILDREN WITH HIGH-GRADE GLIOMA

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BACKGROUND: For pediatric high-grade glioma (pHGG), non-invasive methods for diagnosis and surveillance are needed. Tumors release DNA (tDNA) into cerebrospinal fluid (CSF), allowing for detection of tumor-associated mutations by CSF sampling. We hypothesized that direct, electronic analysis of tDNA with a novel, hand-held platform (Oxford Nanopore MinION) could quantify patient-specific CSF tDNA variant allele fraction (VAF) with improved speed and limit of detection compared to established methods. METHODS: We integrated required multi-timepoint (0, 2, and 6 months) correlate lumbar punctures (LP) in two ongoing pHGG clinical trials. Using Nanopore technology, we performed amplicon-based PCR on CSF tDNA for recurrent mutations from patient samples (n=19) and normal controls. VAF were determined via MinKNOW, Guppy, MiniMap2, and Integrated Genome Browser. RESULTS: Nanopore CSF tDNA demonstrated improved sensitivity (91%) when compare to NGS sequencing (50%). Nanopore analysis of serially diluted CSF sample demonstrated significantly lower limit of detection (attomolar) than typical NGS sample requirement (nanomolar). H3K27M mutation was reliably detected with 1,000x depth sequencing, which was achieved in less than 15 minutes of sequencing after amplification. Multiplexed Nanopore analysis of *H3F3A* and *HIST1H3B* was employed when H3 status was unknown. Serial CSF tDNA analysis confirmed multi-gene (*H3F3A* K27M, *PIK3CA*, and *TP53*) molecular remission in a 17-year-old with thalamic diffuse midline glioma that correlated with sustained clinical response to ONC201 (14 months and ongoing). CONCLUSIONS: Use of a hand-held, electronic DNA analysis platform allows quantification of multi-gene molecular response with improved speed and limit of detection in the CSF of children with high-grade glioma.

DIPG-10. OPTIMAL HDAC INHIBITION IN DIFFUSE INTRINSIC PONTINE GLIOMA

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As the majority of diffuse intrinsic pontine glioma (DIPG) have H3K27M mutations, epigenetic-targeting agents have been studied, though evaluations have been limited by their model systems, untranslatable drug concentrations, and/or evasive mechanisms of action. To develop a more translational model, we used biopsy samples from newly diagnosed DIPG patients to create treatment-naïve *in vitro* and *in vivo* models (molecular aberrations in parentheses), including PBT-09FH (*H3FA3*, *PI3KCA*), PBT-22FH (*H3F3A*,