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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*,

TP53), PBT-24FH (*PMS2*), and PBT-27FH (*HIST1H3B, TP53, NTRK2*). Models demonstrated radiation-resistance similar to the patient from whom the culture was generated, supporting the models' relevance (e.g. cell viability after 8 Gy was 36%, 81%, 71%, and 61% in PBT-09FH, -22FH, -24FH, and -27FH, respectively, compared to 7% in the medulloblastoma model MED-411FH). We evaluated cell viability and apoptosis following treatment with a panel of HDAC inhibitors, identifying the low nanomolar IC_{50} of quisinostat (~50 nM) and romidepsin (~5 nM). While RNA expression changes induced by 100 nM panobinostat and quisinostat included shared overexpression of the top 20/25 genes (e.g. *FSTL5*, *ITIH5*) and shared downregulation of the top 22/25 (e.g. *GPR37L1*, *HEPACAM*), only 9/25 were downregulated by panobinostat, quisinostat, and romidepsin (e.g. *C21orf62, IFIT2*), identifying these as potential vulnerabilities or biomarkers of lethal HDAC inhibition. Mass-spectrometry (LC-MS) demonstrated panobinostat as the greatest acetylator of cortactin, potentially related to thrombocytopenia. While PBT-09 flank models demonstrated quisinostat's on-target acetylation and efficacy, orthotopic xenograft models did not, supporting our model's intact blood-brain barrier and emphasizing the need for CNS penetrant versions of potentially efficacious agents.

DIPG-11. A PHASE I DOSE ESCALATION STUDY OF BXQ-350 IN CHILDREN AND YOUNG ADULTS WITH RELAPSED SOLID TUMORS

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BXQ-350 is a novel agent composed of the multifunctional, lysosomal activator protein Saposin C (SapC) and dioleoyl- phosphatidylserine (DOPS). BXQ-350 demonstrated antitumor effects *in vitro and in vivo*. Many tumors, including diffuse intrinsic pontine glioma (DIPG), and cells of tumor vasculature have aberrantly-exposed PS-rich domains on the cell surface. BXQ-350 is an anti-tumor agent in development from Bexion Pharmaceuticals, Inc. that selectively targets tumor cell PS, particularly those translocated to the outer leaflet of the plasma membrane in tumor cells. BXQ-350 activates and participates in various cellular processes, including apoptosis and necrosis, and may also exhibit novel mechanisms leading to cell death that require further investigation. An adult Phase I trial with BXQ-350 completed enrollment in 2019 having dosed 86 recurrent solid tumor patients, including glioblastoma, with only one serious infusion-related reaction. The highest planned dose of 2.4 mg/kg was achieved and seven patients remain on study with multiple cases demonstrating an objective response. A Phase I pediatric dose escalation trial in recurrent solid tumors, including central nervous system (CNS) tumors, also completed enrollment in 2019. The highest planned dose of 3.2 mg/kg was achieved and there have been no BXQ-350 related serious adverse events. Eight patients (7 CNS and 1 non-CNS) completed at least one cycle with one DIPG patient completing cycle five. A pediatric Phase I trial in newly diagnosed DIPG and diffuse midline glioma (DMG) is planned for 2nd quarter 2020.

DIPG-12. TARGETING EPIGENETIC MODIFIERS TO INDUCE IMMUNE SIGNALING IN DIPG

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DIPG is a universally fatal pediatric brainstem tumor with no effective therapy. Recent work has shown that over 80% of DIPG cases harbor the H3K27M mutation leading to global loss of the repressive H3K27 trimethylation mark, global DNA hypomethylation, and a distinct gene expression signature. We sought to exploit epigenetic vulnerabilities in DIPG through the use of DNA methyltransferase inhibitors and histone deacetylase (HDAC) inhibitors. We find that treatment with low-dose 5-aza-2'-deoxycytidine (decitabine), alone and in combination with HDAC inhibitors, elicits profound genome-wide demethylation in DIPG patient-derived neurosphere cell lines, impairs proliferation, and induces apoptosis. We show that this treatment induces immune activation, with induction of type I interferon signaling, increased expression of major histocompatibility complexes, and expression of tumor antigens. These results suggest that the immunogenicity of DIPG may be modulated by epigenetic therapies, suggesting the possibility of novel combination approaches to immunotherapy of DIPG in the future.

DIPG-13. TARGETING HYPOXIA AND MITOCHONDRIA WITH REPURPOSED METABOLIC DRUGS AS AN APPROACH TO RADIOSENSITIZATION FOR DIFFUSE INTRINSIC PONTINE GLIOMAS (DIPG)

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DIPG is the leading cause of brain tumor-related death in children. Currently, radiation is the only treatment that offers transient benefit. Compared to normal brain tissue, DIPGs are hypoperfused with tumors being exposed to hypoxia, a potent barrier to effective radiotherapy. Biguanides are hypoglycemic agents that can reduce the oxygen consumption rate (OCR) in mitochondria, thereby reducing hypoxia. Our previous study has shown that metformin significantly improves the radiosensitivity of DIPG and extends survival in a patient-derived xenograft (PDX) model. In the present study, phenformin, a second biguanide derivative, demonstrated even greater anti-DIPG activity and radiosensitising effect *in vitro*. As a single agent, phenformin dose-dependently inhibited OCR and increased extracellular acidification rate (ECAR). Low-dose phenformin reduced mitoATP/glycoATP ratio, whereas high doses significantly suppressed net ATP production. To attenuate the phenformin-induced ECAR, phenformin was combined with dichloroacetate (DCA), a clinically relevant pyruvate dehydrogenase kinase inhibitor that can suppress the elevated glycolytic rate of cancers. This combination significantly blocked the phenformin-induced ECAR and killed DIPG cells synergistically by inducing apoptosis, DNA damage and metabolic catastrophe. Moreover, protein expression of HIF-1α and c-Myc, two master regulators that collaboratively enhance the metabolic capacity of tumor cells through increased glycolysis thereby contributing to radioresistance, were also suppressed by phenformin-DCA treatment *in vitro*. This combination therapy upregulated genes inhibiting cell proliferation while downregulating genes for DNA repair. The triple combination of phenformin, DCA and irradiation demonstrated the most potent efficacy *in vitro* and is currently being tested in our PDX cohort *in vivo*.

DIPG-14. TARGETING POLO-LIKE KINASE 1 IN COMBINATION WITH KEY ONCOGENIC DRIVERS IN DIPG: FROM SINGLE AGENT TO COMBINATION STRATEGIES

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Diffuse Intrinsic Pontine Glioma (DIPG) are devastating paediatric brainstem tumours. Loss of function mutations in DIPG decrease genetic stability and impair DNA damage response pathways promoting tumourigenesis. Polo-like Kinase 1 (PLK1) is a pivotal controller of cell growth, regulating key intermediaries of DNA replication, homologous repair, the cell cycle and cell division. We have found DIPG cultures consistently overexpress PLK1 with inhibition resulting in decreased tumour cell growth, heightened cell cycle arrest and apoptosis. Single agent treatment using PLK1 inhibitors unprecedentedly doubled the median survival of animals harbouring DIPG tumours. Through gene expression analysis, we've showed PLK1 inhibition affected multiple pathways which control the cell cycle, cell death regulation, microtubule organization and regulation of cell migration. We found these pathways of differentially expressed genes were significantly enriched for known targets of both E2F1 and E2F4. Analysis of gene expression and proteomic studies also revealed PLK1 inhibition decreased the activation and expression of key tumour promoting mediators within multiple phases of the cell cycle, decreased expression of tumour promoters including MYC and the PI3K/mTOR pathway and reactivated tumour suppressors p53 and PTEN. Assessing these changes in the treated transcriptome and proteome, we aim to develop multiple potentially translatable combination treatment strategies for DIPG. We have performed mechanistic studies and identified synergism with PLK1 inhibitors and the epigenetic regulator panobinostat, bet/bromodomain inhibitor JQ1, dual PI3K/mTOR inhibitor bimiralisib and PI3K inhibitor BKM120. Finally, we found PLK1 inhibitors act as potent radiosensitizers, enhancing the therapeutic effects of radiotherapy *in vitro* and *in vivo*.

DIPG-15. POLYAMINE PATHWAY INHIBITION IS A POTENT NOVEL THERAPEUTIC STRATEGY AGAINST DIFFUSE INTRINSIC PONTINE GLIOMA

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