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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DIPG is an aggressive paediatric brainstem tumour, with a median survival of less than 1 year. Polyamines are intracellular polycations that control important aspects of cell growth and are often upregulated in cancer. Difluoromethylornithine (DFMO) is an FDA-approved inhibitor of the enzyme ornithine decarboxylase (ODC1) which is a key driver of polyamine synthesis. We investigated the efficacy of polyamine pathway inhibitors as a therapeutic strategy against DIPG. We found high expression levels of synthetic enzymes in the polyamine pathway in primary patient samples and cultures. Using cytotoxicity and clonogenic assays, we found that DFMO inhibited the proliferation of DIPG neurospheres. However, DIPG cells compensated for DFMO inhibition by increasing expression of the polyamine transporter SLC3A2. Gene expression analysis showed that the polyamine transporter, SLC3A2, was significantly overexpressed in DIPG compared with all other high-risk childhood cancers. Addition of polyamine transporter inhibitor AMXT 1501 to DFMO led to synergistic inhibition of DIPG proliferation. Consistent with the *in vitro* results, the combination treatment significantly prolonged the survival of mice bearing 3 different DIPG orthografts with 2/3 of the animals surviving up to 160 days. Addition of irradiation further improved the survival of mice treated with DFMO and AMXT 1501. Our results suggest that DIPG tumours are exquisitely sensitive to polyamine inhibitors and that dual blockade of polyamine synthesis and transport is a promising novel therapeutic strategy. AMXT 1501 is currently in clinical development for adult cancers (NCT03536728). A clinical trial for DIPG patients is planned through the CONNECT consortium.

DIPG-16. COMBINATION OF ARGININE DEPLETION AND POLYAMINE INHIBITION AS AN ANTICANCER STRATEGY FOR DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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DIPG is an aggressive pediatric brainstem tumor, with a median survival below 12 months. Tumor cells are dependent upon arginine, a semi-essential amino acid, metabolised by arginase enzymes into ornithine, a pivotal precursor to the polyamine pathway. Polyamines, frequently upregulated in cancer, are intracellular polycations controlling key biological processes – the inhibition of which we have previously shown to be highly efficacious in preclinical DIPG models. Pegylated arginase (BCT-100) has recently been shown to significantly delay tumor development, prolonging survival of neuroblastoma-prone *Th-MYCN* mice. This study investigated the effects of arginine depletion therapy as a single agent and in combination with polyamine pathway inhibitors in DIPG. We found that ARG2, the gene encoding for arginase II, is expressed significantly more highly in DIPG tumors compared to normal brain. Arginine depletion via BCT-100 reduced DIPG cell proliferation and colony formation in patient-derived cell lines. Using orthotopic patient-derived xenograft models of DIPG, we found that frequent dosing of BCT-100 (4x/week) significantly delayed tumor development and increased the survival of the mice ($p<0.0001$). DFMO is an FDA-approved inhibitor of the enzyme ornithine decarboxylase, a key driver of polyamine synthesis. The combination of BCT-100 with DFMO led to significant enhancement in DIPG survival ($p<0.005$ compared to single agent treatments). Triple combination therapy with addition of the polyamine transport inhibitor AMXT-1501 led to a potent and profound improvement in survival. These data show that arginine depletion therapy using BCT-100 combined with dual polyamine inhibitory agents represents a potentially exciting new approach for the treatment of DIPG.

DIPG-17. BIOPSY-PROVEN DIFFUSE MIDLINE GLIOMA IN ADOLESCENTS AND YOUNG ADULTS

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INTRODUCTION: Diffuse midline glioma (DMG) mostly affects young children. The newly-introduced disease entity DMG, H3K27M-mutant uniformly portends poor prognosis, and therefore that in the pons is usually treated based upon radiological diagnosis without histological confirmation. DMG is rarer in adolescents and young adults (AYA), and remains poorly characterized. In this study, we sought to investigate the clinical, pathological, and molecular profiles of DMG in AYA generation. METHODS: Patients of age between 16 and 39 undergoing biopsy at the University of Tokyo Hospital between 2003 and 2019 were included in the study. Clinical data and images were retrospectively reviewed. Genetic analyses were performed in cases with abundant tissues. RESULTS: Ten patients included 8 brainstem and 2 thalamic DMG. The median age was 25 years (range, 19–38). Pathological diagnosis was DMG, H3K27M-mutant in 3 patients, glioblastoma, IDH-mutant in 1, anaplastic astrocytoma, IDH-wildtype in 4, diffuse astrocytoma, IDH-mutant in 1, and diffuse astrocytoma, IDH-wildtype in 1. Genetic analyses detected *H3F3A*-K27M mutation in 2, *HIST1H3B*-K27M mutation in 1, *IDH1*-R132H mutation in 1, and *IDH1*-R132S mutation in 1. With a median follow-up of 23 months (range, 2–61), only 3 patients died 29–61 months after diagnosis, and the remaining 7 patients survived for 2–59 months. Neither *IDH1* mutation nor H3K27M mutation was associated with survival in this series. CONCLUSION: Survival of AYA patients with DMG was seemingly variable with some long survivors. H3K27M mutation was present in a subset of patients. A further study is warranted to correlate molecular profile with clinical pictures including patient survival.

DIPG-18. IDENTIFICATION OF TARGETABLE PATHWAY DEPENDENCIES IN DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse Intrinsic Pontine Glioma (DIPG) is a highly aggressive paediatric brainstem tumour with a dismal prognosis. Recurrent heterozygous mutations (p.K27M) in Histone H3 variant genes have been identified in the majority of DIPG cases. While the exact mechanism of H3K27M's function is poorly understood, evidence suggests a role for epigenetic dysregulation in disease pathogenesis. This study aims to use functional genomics to identify novel therapeutic dependencies in H3K27M DIPG. DIPG drug sensitivity screening was carried out in twelve established and validated patient derived cell lines (10 H3.3K27M and 2 Wt) using an FDA approved drug library containing 1480 compounds. Highly prevalent targets identified from this screen include HDAC, microtubule, proteasome and CDK inhibitors. Additionally, a custom pooled CRISPR knockout library of druggable targets (300 genes, 1200 guide RNAs) was used to identify key DIPG cell survival pathways. To date five DIPG cell lines (1 Wt; 1 H3.1; 3 H3.3) have undergone screening. Knockdown of known DIPG driver genes (*TP53; PDGFRA*; *PIK3CA* and *PIK3CR1*) resulted in reduced cell viability, consistent with their proposed function and validating knockout screen utility. Preliminary data demonstrates Wt and H3K27M DIPGs cluster independently based on genes required for survival, suggesting differing tumorigenesis mechanisms and the potential for therapeutically targeting genotype specific pathways. Correlation of parallel drug screen and RNA-seq data will potentially reveal H3-dependent pathways for therapeutic exploitation. Collectively, we show a functional genomics approach is able to identify genotype-specific pathway dependencies in DIPG, paving the way for molecularly informed personalized therapies for patients.

DIPG-19. TARGETING ATM MUTATION IN METASTATIC DIFFUSE MIDLINE GLIOMA – A CASE OF SUSTAINED RESPONSE USING PARP INHIBITOR

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Diffuse midline glioma (DMG) with H3.3K27M mutation is associated with an extremely poor prognosis, with a median survival of 10 to 12 months. Radiation remains the standard of care however there is no established curative therapy available. We describe a patient diagnosed with a diffuse intrinsic pontine glioma at 5 years of age by clinical and radiological criteria. He was treated with focal radiation 59Gy which resulted in reduction in size of the tumour, and partial improvement of T2 changes on MRI. At 18 months post diagnosis, the patient developed metastatic recurrence at the anterior fornix. This was biopsied and histopathology demonstrated a high grade glioma. Next generation sequencing revealed a H3F3A K27M mutation, and an ATM R3008H mutation. He received whole ventricular radiation 36Gy and boost to the lesion to 45Gy, followed by Olaparib 135mg/m2/day twice daily. He remains in radiological remission 20 months post metastatic relapse and has no organ toxicity to Olaparib. CONCLUSION: H3.3K27M and ATM co-segregating muta-