TP53), PBT-24FH (PM25), 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 IC50 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 2025 genes (e.g. FSTL1, ITIH5) and shared downregulation of the top 2225 (e.g. GPR37L1, HEPACAM), only 925 were downregulated by panobinostat, quisinostat, and romidepsin (e.g. C21or62, 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 capacity to 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**

Bhuvana Setty1, Timothy Crape1, Mariko DeWiere-Schottmiller1, Richard Curry2, and Mohamed Abdel-Baki3, 1 Nationwide Children’s Hospital, Columbus, OH, USA; 2 Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA; 3 Bexion Pharmaceuticals, Covington, KY, USA

BXQ-350 is a novel agent composed of the multifunctional, lysosomal activator protein Sapsoin C (Sac) and diacetyl-, phosphatidylethanolamine (DPE), Bexion’s dual anti-tumor capacity of tumor demethylation 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 specifically targets tumor cell PS, particularly those translated 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. A Phase I trial with BXQ-350 completed enrollment in 2019 having dosed 86 recurrent solid tumor patients, including glomblastosarcoma, 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 trial with BXQ-350 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. Two patients enrolled 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**

Ashley Tetens1, Allison Martin1, Amie Arnold1, Orlandi Novak1, Charles Eberhart2, Andrew Fenberg1, Eric Raabe1, and Michael Koldobski3, 1 Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2 Albert Einstein College of Medicine, the Bronx, NY, USA

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, cell cycle arrest, 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)**

Han1,2,3, Cecilia Chang1, Harriet Gee1,3, Kelly McKelvey1,2, Geraldine O’Neill1,2, Victoria Prior1,3, Anneke Blackburn1, and Eric Hau1,2

1Discipline of Child and Adolescent Health, 2Department of Radiation Oncology, Crown Prince Family Cancer Centre, Westmead Hospital, Westmead, NSW, Australia; 3Bill Walsh Translational Cancer Research, Kolling Institute, St Leonards, NSW, Australia; 4Children’s Cancer Research Unit, Children’s Hospital at Westmead, Westmead, NSW, Australia; 5Discipline of Child and Adolescent Health, Faculty of Medicine and Health, University of Sydney, Camperdown, NSW, Australia; 6The John Curtin School of Medical Research, The Australian National University, Acton, ACT, Australia

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 glucose dehydrogenase kinase inhibitors 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 through increased glycolysis and in vivo. These pathways were identified in untreated DIPG samples, whereas in phenformin-treated samples, HIF-1α and c-Myc were downregulated, indicating a potential role for biguanides in treating DIPG.

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

Laura Franshaw1, Elisha Hayden1, Swapna Joshi1, Jie Liu1, Anahid Ehteda1, Chi Nam Ignatius Pang1, Mario Ted1, and David S. Ziegler1, 1 Children’s Cancer Institute, Randwick, NSW, Australia; 2 School of Biotechnology and Biomolecular Sciences, University of New South Wales, Randwick, NSW, Australia; 3 Kids Cancer Centre, Sydney Children’s Hospital, Randwick, NSW, Australia

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 unpredictably 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 have identified multiple differentially expressed markers whose identification and validation could be used as potential drug targets or combination therapies 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 PLK1 inhibitor BKM120. Finally, we ligated PLK1 inhibitors as potent radiosensitzers, 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**

Aamnah Khan1, Laura Gamble1, Dannielle Upton1, Denise Yu1, Anahid Ehteda1, Ruby Pandher1, Chelsea Mayo1, Mark Burns1, Murray Norris1, Michelle Haber1, Mario Ted1, and David S. Ziegler1, 1 Centre for Cancer Research, Westmead Institute for Medical Research, Westmead, NSW, Australia; 2 Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Camperdown, NSW, Australia; 3 Department of Radiation Oncology, Crown Prince Family Cancer Centre, Westmead Hospital, Westmead, NSW, Australia; 4 Bill Walsh Translational Cancer Research, Kolling Institute, St Leonards, NSW, Australia; 5 Children’s Cancer Research Unit, Children’s Hospital at Westmead, Westmead, NSW, Australia; 6 Discipline of Child and Adolescent Health, Faculty of Medicine and Health, University of Sydney, Camperdown, NSW, Australia; 7 The John Curtin School of Medical Research, The Australian National University, Acton, ACT, Australia

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 unpredictably 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 have identified multiple differentially expressed markers whose identification and validation could be used as potential drug targets or combination therapies 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 PLK1 inhibitor BKM120. Finally, we ligated PLK1 inhibitors as potent radiosensitzers, enhancing the therapeutic effects of radiotherapy in vitro and in vivo.
DIPG is an aggressive paediatric brainstem tumour, 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 arginine (BCT-100) has recently been shown in further preclinical studies to improve the survival of mice treated with DFMO and AMXT 1501. Our studies suggest that DIPG tumours are excessively 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-18. IDENTIFICATION OF TARGETABLE PATHWAY DEPENDENCIES IN DIFFUSE INTRINSIC PONTINE GLIOMA**

Sarah Parackal1, Wai Chin Chong1, Gabrielle Bradshaw2, Claire Sun1, Paul Daniel1, Enola Roussel2, Samantha Jayasekara2, Duncan Crambie1, Ron Firestein1, and Jason Caan2, 3
1Centre for Cancer Research, Hudson Institute, 2Department of Medical Research, 3Molecular Translational Science, School of Clinical Sciences, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, VIC, Australia, 2Centre for Cancer Research, Hudson Institute of Medical Research, 3Department of Genetics, School of Biological Sciences, Faculty of Science, Monash University

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.K27M and 2 Wt) using an FDA approved drug library containing 1480 compounds. High-confidence targets identified from this screen include HDAC, microtubule, proteasome and CDK inhibitors. Additionally, a custom pooled CRISPR knock out 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; PDGFRα; PIK3CA and PIK3R1) resulted in reduced cell viability, consistent with their proposed function and validating knockdown screen utility. Preliminary data demonstrates Wt and H3K27M DIPG clones independently based on genetic screening differ in drug sensitivity, proliferation and survival, demonstrating 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 that functional genomics is a more high fidelity approach which is able to identify genotype-specific pathway dependencies in DIPG, paving the way for molecularly informed personalized therapies for patients.