Phenotypic assessment of the models *in vitro* by high-throughput imaging demonstrated significantly increased invasion and migration in association with either KMT5B or KMT5C loss, but not both. Quantitative proteomic assessment of the secretome identified factors by which a minority of KMT5B-deficient cells may signal to promote motility of the neighbouring populations. These data suggest a previously unrecognised trans-histone (H4/H3) interaction in DIPG cells with a potentially profound effect on their diffusely infiltrating phenotype.

DIPG-64. INTERNATIONAL PRECLINICAL DRUG DISCOVERY AND BIOMARKER PROGRAM INFORMING AN ADOPTIVE COMBINATORIAL TRIAL FOR DIFFUSE MIDLINE GLIOMAS

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INTRODUCTION: DMG-ACT (DMG- multi-arm **A**daptive and **C**ombinatorial **T**rial) aims to implement a highly innovative clinical trial design of combinatorial arms for patients with diffuse midline gliomas (DMGs) at all disease stages that is adaptive to pre-clinical data generated in eight collaborating institutions. The goals of the team are to: i) rapidly identify and validate promising drugs for clinical use, and ii) predict biomarkers for promising drugs. METHODS: In vitro (n=15) and in vivo (n=8) models of DMGs across seven institutions were used to assess single and combination treatments with ONC201, ONC206, marizomib, panobinostat, Val-083, and TAK228. In vivo pharmacokinetic assays using clinically relevant dosing of ONC201, ONC206, and panobinostat were performed. Predictive biomarkers for ONC201 and ONC206 were identified using extensive molecular assays including CRISPR, RNAseq, ELISA, FACS, and IHC. RESULTS: Inhibitory concentrations (IC_{50}) were established and validated across participating sites. In vivo validation of single and combination drug assays confirmed drug efficacy as increased survival for: ONC201 (p=0.01), ONC206 (p=0.01), ONC201+ONC206 (p=0.02), and ONC201+panobinostat (p=0.01). Marizomib showed toxicity in murine/zebrafish PDXs models. Murine pharmacokinetic analysis showed peak brain levels of ONC201 and ONC206 above pre-clinical IC_{50}. Molecular testing and analyses of existing drug screen across 537 cancer cell lines validated mitochondrial stress and ATF4 as the main targets induced by ONC201/6. CONCLUSION: Thorough preclinical testing in a multi-site laboratory setting is feasible and identified ONC201 in combination with ONC206 as promising therapeutics for DMGs. Preclinical and correlative-clinical studies are ongoing.

DIPG-66. FEASIBILITY AND APPLICABILITY OF MOLECULAR GUIDED THERAPY IN HIGH GRADE GLIOMA/DIFFUSE MIDLINE GLIOMA: RESULTS FROM BEAT CHILDHOOD CANCER NMTRC-009 MOLECULAR GUIDED THERAPY STUDY

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High grade gliomas/diffuse midline gliomas (HGG/DMG) historically have a poor prognosis with an overall survival of less than 20% at 5 years. The pathophysiology is under close investigation across the world in efforts to understand this tumor type with aims of increasing effective treatment options. We present our results on the feasibility and outcomes of patients treated on our Molecular Guided Therapy study. Tumor samples were analyzed with whole exome (DNA) and RNA sequencing. Three drug matching algorithms were utilized to generate a report that was reviewed at a multi-institutional tumor board meeting, culminating in a proposed treatment protocol. Eleven patients enrolled, but one did not complete cycle 1 of therapy due to progression of disease, thus ten patients (6-HGG, 4-DMG) were evaluable and received at least 2 cycles of therapy. Time to reports generated and tumor board assembly was (median) 18 and 24 days, respectively. Secondary goals included evaluation of efficacy. Responses showed 50% of patients with stable disease or better at 2 cycles of therapy, but these were temporary with median time to progression of 81 days. In conclusion, we determined that it is feasible to collect individual biological DNA and RNA sequencing information to offer patients individualized treatment plans for this devastating group of diseases. Though data is not statistically significant, we show that there is a suggestion of efficacy in this approach to treatment for patients, indicating a need to expand on this treatment approach with individualized medicine.

DIPG-68. ALPHA-THALASSEMIA X-LINKED MENTAL RETARDATION PROTEIN (ATRX) LOSS-OF-FUNCTION IN A MOUSE MODEL OF DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse Intrinsic Potine Glioma (DIPG) is a rare pediatric brain tumor for which no cure or efficacious therapies exist. Previous discoveries have revealed that, DIPG harbors distinct genetic alterations, when compared with adult high-grade glioma (HGG) or even with non-DIPG pediatric HGGs. ATRX alteration is found in 9% of clinical cases of DIPG, and significantly overlaps with H3.3K27M mutation and p53 loss, the two most common genetic changes in DIPG, found in 80% and 77% clinical cases, respectively. Here we developed genetically engineered mouse model of brainstem glioma using the RCAS-Tv-a system by targeting PDGF-B overexpression, p53 loss, H3.3K27M mutation and ATRX loss-of function to Nestin-expression brainstem progenitor cells of the neonatal mouse. Specifically, we used Nestin-Tv-a; p53 floxed; ATRX heterozygous female and Nestin-Tv-a; p53 floxed; ATRX floxed male breeders, generated offsprings with ATRX loss of function (n=18), ATRX heterozygous females (n=6), and ATRX WT (n=10). Median survial of the three groups are 65 days, 88 days and 51 days, respectively. Also, ATRX null mice is lower in tumor incidence (44.4%), compared with ATRX WT (80%). We evaluated the pathological features of DIPG with or without ATRX alteration, RNA-seq is performed to identify differentially expressed genes between ATRX WT and loss-of-function. In conclution, this study generated the first genetically modified mouse model studying ATRX loss-of-function in DIPG, and suggested that ATRX loss-of-function in DIPG may slow down tumorigenesis and decrease tumor incidence.

DIPG-70. DISORDERED DNA METHYLATION IN DIPG UNDERLIES PHENOTYPIC PLASTICITY

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Diffuse intrinsic pontine glioma (DIPG) is a childhood brainstem tumor with a dismal prognosis and no effective treatment. Recent studies point to a critical role for epigenetic dysregulation in this disease. Nearly 80% of DIPGs harbor mutations in histone H3 encoding replacement of lysine 27 with methionine (K27M), leading to global loss of the repressive histone H3K27 trimethylation mark, global DNA hypomethylation, and a distinct gene expression profile. However, a static view of the epigenome fails to capture the plasticity of cancer cells and their gene expression states. Recent studies across diverse cancers have highlighted the role of epigenetic variability as a driving force in tumor evolution. Epigenetic variability may underlie the heterogeneity and phenotypic plasticity of DIPG cells and allow for the selection of cellular traits that promote survival and resistance to therapy. We have recently formalized a novel framework for analyzing variability of DNA methylation directly from whole-genome bisulfite sequencing data, allowing computation of DNA methylation entropy at precise genomic locations. Using these methods, we have shown that DIPG exhibits a markedly disordered epigenome, with increased stochasticity of DNA methylation localizing to specific regulatory elements and genes. We evaluate the responsiveness of the DIPG epigenetic landscape to pharmacologic modulation in order to modify proliferation, differentiation state, and immune signaling in DIPG cells.

DIPG-71. SELECTIVE HDAC INHIBITOR RG2833 INDUCES DIPG CELL DEATH VIA DOWNREGULATION OF THE NFκB PATHWAY

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Histone deacetylase (HDAC) inhibitor panobinostat demonstrated activity against diffuse intrinsic pontine glioma (DIPG) *in vitro*, but its efficacy *in vivo* was limited by toxicity and poor blood brain barrier penetration. RG2833 (RGFP109) is a selective HDAC1/3 inhibitor that has established brain penetration. In clinical trials, the Cmax (plasma) of RG2833 was 32uM. RG2833 demonstrated cytotoxicity against temozolomide-resistant