Phenotypic assessment of the models in vitro by high-throughput imaging demonstrated significantly increased invasion and migration in association with either KMT3B or KMT3C loss, but not both. Quantitative proteomic analysis identified the secretome identified factors by which a majority of KMT3B-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 infiltratively phenotypic.

DIPG-64. INTERNATIONAL PRECLINICAL DRUG DISCOVERY AND BIOMARKER PROGRAM INFORMATION ON AN ADOPTIVE COMBINATORIAL TRIAL FOR DIFFUSE MIDLINE GLIOMAS
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INTRODUCTION: DMG-ACT (DMG- multi-arm Adaptive and Combinatorial Trial) 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 the MATCH trial. Specific goals of the DMG- ACT trial 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 agent, combination treatments with ONC201, ONC206, tempol, 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 (IC50) 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). Marzomib showed toxicity in murine/zebrafish PDx models. Murine pharmacokinetic analysis showed peak brain levels of ONC201 and ONC206 above pre-clinical IC50. Molecular testing and analyses of existing drug screen across 537 cancer cell lines validated the utility of the oncotargeting strategy, with the most promising being identified by ONC201/6. CONCLUSION: Thorough preclinical testing in a multi-site combinatorial cohort has identified specific targets and validated across participating sites for DMGs.

DIPG-66. FEASIBILITY AND APPLICABILITY OF MOLECULAR GUIDED THERAPY IN HIGH GRADE GLIOMA/DIFFUSE MIDLINE GLIOMA: RESULTS FROM BEAT CHILDHOOD CANCER NMRCT-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 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, the data did not completely match protocols 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 the difficult to treat group of studies. Therefore, data from our pilot study suggest, 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 Pontine Glioma (DIPG) is a rare pediatric brain tumor for which no cure or effective 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 DIPGs, and significantly overlapped with H3.K27M 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 R23-the ATRX loss of function in Nestin-expressing brainstem progenitor cells of the neonatal mouse. Specifically, we used Nestin-Tv-a; p53 floxed; ATRX heterozygous females and Nestin-Tv-a; p53 floxed; ATRX floxed mice to establish a mouse model of DIPG with ATRX loss-of-function (n=18), ATRX heterozygous females (n=6), and ATRX WT (n=10). Median survival 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 was performed to identify differentially expressed genes between ATRX WT and loss-of-function. In conclusion, this study generated the first genetically modified mouse model studying DIPG with ATRX alteration, and validated the ATRX loss-of-function in DIPG may slow down tumor growth 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. Prior 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 H3K27m 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.