Abstracts

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 several secretome identified factors by which a minority of KMT3B-deficient cells may signal to promote motility of the neighboring populations. These data suggest a previously unrecognized 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 THERAPY FOR DIFFUSE MIDLINE GLIOMAS
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INTRODUCTION: DMG-ACT (DMG- multi-arm Adaptable 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 during the molecular testing and analyses of existing drug screen across 537 cancer cell lines. The primary goal is to 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 panobinostatin 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+panobinostatin (p=0.01). Marizomib showed toxicity in murine/neuroblast 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 were used to identify ARID1A and ARID2 loss of function in DIPG may slow down tumorigenesis and decrease tumor incidence.

DIPG-66. FEASIBILITY AND APPLICABILITY OF MOLECULAR GUIDED THERAPY IN HIGH GRADE GLIOMA/DIFFUSE MALIGNANT GLIOMA: RESULTS FROM BEAT CHILDHOOD CANCER NTMRC-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, uptake was not complete 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 specific tumor type. Data from this study in DIPG, and suggested that ATRX loss-of-function, 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 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.

1ATRX deletion is found in 93% of clinical cases of DIPGs, and significantly overlaps 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 RCAS-TA-Alexa retrovirus. We evaluated the biological effects of ATRX loss-of-function on brainstem expression in cell lines. Specifically, we used Nestin-Tv-a; p53 floxed; ATRX heterozygous females and Nestin-Tv-a; p53 floxed; ATRX homozygous ENU mice. ATRX deletion resulted in increased glioma burden, increased survival, and decreased glioma burden, increased survival, and decreased glioma burden, increased survival, and decreased glioma burden.

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 DIPG harbor mutations in histone H3 encoding replacement of lysine 27 with methionine (K27M), leading to global loss of the repressive H2AK119 mono and di acetylation from histone H3K27 trimethylation mark, 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 NFKB 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 (RGP109) is a selective HDAC1/3 inhibitor that has established brain penetration. In clinical trials, the Cmax (plasma) of RG2833 was 12μM. RG2833 demonstrated cytotoxicity against temozolomide-resistant