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BACKGROUND: Diffuse intrinsic pontine gliomas (DIPGs) are lethal pediatric brain tumors with no curative therapies. Inhibitor of DNA binding (ID) proteins are key regulators of gene differentiation during embryogenesis. Previous work has shown that *H3F3A* and *ACVR1* mutations increase ID1 expression in cultured astrocytes, but this has not been validated in human DIPG, nor has the regulation and targetability of ID1 been explored in DIPG. RESULTS: Analysis of post-mortem tissue and multiple human datasets showed ID1 to be elevated in DIPG, and to correlate with reduced survival. In a multi-focal autopsy of a DIPG case, we also found ID1 expression to be heterogeneous and to correlate with tumor invasion. Chromatin immunoprecipitation qPCR (ChIP-qPCR) revealed elevated H3K27ac and low H3K27me3 at ID1 regulatory regions (enhancers/promoters) in DIPG tissue compared to normal brain, regardless of H3 or *ACVR1* mutation status. Analysis of publicly-available ISH and ChIP-sequencing data of developing murine brains revealed H3K27ac at ID1 enhancers to be elevated in the prenatal hindbrain compared to prenatal forebrain and midbrain, and all postnatal brain regions. ID1 shRNA-mediated knockdown of primary human H3K27M DIPG cells (DIPG007) significantly reduced invasion and migration. We also treated DIPG007 cells with cannabidiol (CBD) and found reduced viability at clinically relevant dosing (IC50=2.4 uM) with dose-dependent reduction in ID1 protein. CONCLUSIONS: These findings indicate that a multifactorial (genetic and regionally-based) epigenetic upregulation of *ID1* drives DIPG invasiveness and is targetable with CBD. ID1 knockdown and CBD treatment experiments in murine models of DIPG are ongoing.

DIPG-60. PILOT STUDY OF CIRCULATING TUMOR CELLS IN PEDIATRIC HIGH GRADE BRAIN TUMORS

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BACKGROUND: Despite its increasing use, circulating tumor cells (CTCs) have not been studied in pediatric brain tumors. METHODS: Cell surface vimentin (CSV) is a marker for CTC detection. We developed an automated CSV-based CTC capture method for pediatric brain tumor using the Abnova Cytoquest platform. PBMCs isolated from blood samples from 52 brain tumor patients were processed to isolate CSV^+ CTCs. Captured cells were then stained for CSV and CD45 and scanned to determine the number of CTCs. DIPG samples were additionally examined for H3K27M expression on CSV^+ cells. Long term cancer survivors were used as a control cohort. RESULTS: 86.4% of all the samples exhibited between 1–13 CSV+ CTCs, with a median of 2 CSV+ CTCs per sample. Using a value of ≥ 1 CTC as a positive result, the sensitivity and specificity of this test was 83.05% and 60.0% respectively. 19 DIPG samples were analyzed and 70% (13 samples) were positive for 1–5 CTCs. Five of these 7 positive CSV+ CTCs DIPG samples were also positive for H3K27M mutations by immunohistochemistry (71%). Mean survival in days for the CTC positive and negative DIPG samples were 114 and 211 days, respectively (p= 0.13). CONCLUSION: This is the first study of CTCs in pediatric CNS tumors using an automated approach. Patients with brain tumors can exhibit CSV+ CTCs within peripheral blood. The use of specific molecular markers such as H3K27M can improve the diagnostic capability of liquid biopsies and may enable future disease assessment for personalized therapy.

DIPG-61. RESCUE REGIMENS AFTER BIOMEDE: POSSIBLE INFLUENCE ON OS ASSESSMENT

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BIOMEDE is a multicentric randomized phase II trial to evaluate in DIPG the OS of patients treated with dasatinib, erlotinib or everolimus. The OS is the result of the first line treatment but it could also be affected by re-irradiation and the second line treatment received after progression, especially in case of a possible crossover outside of the trial. This preliminary analysis focuses on the first patients enrolled at Gustave Roussy (n=37). The median age at diagnosis was 7 years, median interval from diagnosis to progression and median survival after progression were 7 (1–20) and 2 (0–13) months respectively. Initial treatment was everolimus for 13, dasatinib for 20, erlotinib for 4 patients. The most frequent targetable molecular alterations were mTOR pathway in 6, PDGRFA in 4, ACVR1 in 3 patients. Out of the 31 patients who relapsed and were evaluable, 18 and 13 had a median survival < 3 and > 3 months respectively. At relapse patients have received different types of therapies, in 6 cases matching the molecular profile of the tumour obtained by sequencing. At progression, seven patients switched from dasatinib to mTOR inhibitors and 2 patients switched from everolimus to dasatinib. Patients with OS after progression > 3 months had higher rate of reirradiation (77% vs 5%), steroid weaning (69% vs 33%) and Lansky/Karnowsky > 50% (85 vs 67%). Extended results on the entire cohort will be presented. It will be important to consider the distribution of reirradiation to interpret the results of the randomisation on OS.

DIPG-62. PRECLINICAL EVALUATION OF IMIPRIDONE-BASED COMBINATION THERAPIES IN PEDIATRIC H3K27M MUTANT DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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Imipridones induce apoptosis in cancer via p53 independent upregulation of TNF-related apoptosis inducing ligand (TRAIL) pathway and its proapoptotic receptor DR5. ONC201, a first-in-class imipridone, is being evaluated alone and with radiotherapy for children with H3K27M mutant diffuse glioma. We sought to determine if ONC201 and its imipridone analogs (ONC206, ONC212) are synergistic with other chemotherapy agents. Seven patient-derived DIPG cell lines, six H3.3K27M mutant (SU-DIPG-IV, SU-DIPG-13, SU-DIPG-25, SU-DIPG-27, SU-DIPG-29, SF8628) and one H3.1K27M mutant (SU-DIPG-36) were grown in culture and exposed to ONC201, ONC206, and ONC212 alone and in combination with histone de-acetylase inhibitors (HDACi) or etoposide. A dose-dependent response to ONC201, ONC206, and ONC212 was demonstrated in all cell lines, with mean IC50 values of 1.46 μM, 0.11 μM, and 0.03 μM respectively. ONC206 and ONC212 induced apoptosis measured by increased expression of cleaved PARP and ISR by increased expression ATF4. In two cell lines, synergy studies revealed combination indices (CI) < 1 for ONC206 and etoposide, with a best CI of 0.62 in SU-DIPG-IV and 0.46 in SU-DIPG-25. Synergy was also observed between ONC201 and etoposide (CI 0.46) and ONC201 and panobinostat (CI 0.01). Imipridones and analogs were superior to panobinostat and etoposide in triggering apoptosis as measured by sub-G1 phase content. Additional synergy and mechanistic analyses are ongoing and will be reported. Our results suggest that H3K27M mutant DIPG cells demonstrate increased sensitivity to imipridone analogs (ONC206 and ONC212) when compared to ONC201. Combinational strategies with etoposide or HDACi should be considered for clinical translation.

DIPG-63. LOSS OF THE H4 LYSINE METHYLTRANSFERASE KMT5B DRIVES INVASION / MIGRATION BY DEPLETING H3K27ME3 AT LOCI OTHERWISE RETAINED IN H3K27M MUTANT DIPG CELLS

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Diffuse intrinsic pontine glioma (DIPG) and other diffuse midline glioma (DMG) are characterised by K27M mutations in histone H3 variants. The major functional consequence is a global loss of the repressive mark H3K27me3, causing a raft of transcriptional changes promoting tumorigenesis, although certain key loci retain trimethylation, such as *CDKN2A/B*. We recently identified subclonal loss-of-function mutations in the H4 lysine methyltransferase *KMT5B* to be associated with an enhanced invasion/migration, but the mechanism by which this occurred was unclear. Here we show by ChIP-seq using patient-derived subclonal DIPG models and CRISPR-Cas9 depletion that loss of KMT5B (or KMT5C) causes a paradoxical increase in global levels of H4K20me3 in promoters and regulatory regions, only ablated by knocking out both enzymes. Loss of KMT5B alone further causes loss of the majority of otherwise retained H3K27me3 loci in DIPG cells, although *CDKN2A/B* itself was spared. De-repression occurred at bivalent loci marked by H3K4me3 and had elevated gene expression by RNAseq; these were significantly enriched for genes involved in chromatin remodelling and invasion/migration, the latter including *MMP9/MMP24*.

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