to normal caudate tissue, were enriched in cell cycle regulation members. The cluster with worse outcomes had significantly higher activity. Targetable MRs activated in subsets of patients () included TOP2A, CHEK1, CDK2, and EZH2. RNA-seq profiles were generated in two DMG cell lines following perturbation with ~400 oncology drugs, and used to identify drugs that invert MR activity profiles with the OncoTreat algorithm. We identified four pharmacotypes, and predicted sensitivity to HDAC, proteosome, EGFR, MEK, and PI3K inhibitors (), amongst others, consistent with published DMG high-throughput drug screens. To dissect intra-tumor heterogeneity, we measured protein activity from published single-cell RNA-seq profiles of 6 DMG patients, using single-cell based regulatory networks. Preliminary activity-based analysis identified 6 cell states representing distinct stages of differentiation/proliferation, including an oligodendrocyte precursor cell-like proliferative state whose MRs overlapped with bulk data (i.e. TOP2A, CENPF, FOXM1, E2F8, ZWINT, CCNA2), suggesting this as a key regulatory module. We are identifying cell state-specific MR-inverter drugs with OncoTreat to ultimately suggest drugs and drug combinations that collapse the transcriptional program and induce tumor kill for validation in MR-matched preclinical DMG models.

DIPG-58. THERAPEUTIC HDAC TARGETING IN HYPERMUTANT CNS TUMORS

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Diffuse intrinsic pontine glioma (DIPG) is a universally fatal tumor of the pons, most often characterized by mutations in genes encoding histone 3, that led to the classification diffuse midline glioma, H3 K27M-mutant (DMG). However, 15% of DIPG are histone wildtype (H3WT) and can have a hypermutant phenotype, characterized by mutations in DNA mismatch repair that also occur in pediatric high-grade glioma (pHGG). We previously published the preclinical efficacy of quisinostat in DMG. As hypermutant CNS tumors share transcriptional disruption with DMG, we investigated if HDAC inhibition was also therapeutic against hypermutant DIPG and pHGG. We tested PBT-24, a PMS2 mutant, treatment-naïve, patient-derived hypermutant DIPG model, and found similar quisinostat sensitivity compared to other DMG cultures in vitro (IC50 = ~30 nM), as well as significant efficacy in a xenograft flank model (60 day mean tumor size of 1109mm3 [vehicle] vs. 19mm3 [quisinostat-treated]). This in vivo effect was greater than the anti-tumor effect observed in a xenograft flank non-hypermutant DMG model PBT-09 (60 day mean tumor size of 1006mm3 [vehicle] vs. 244mm3 [quisinostat-treated]). To validate the effect of hypermutation on sensitivity to quisinostat, we generated PMS2 knockout (KO) and MSH2 KO isogenic cell lines using the H3WT model VUMC-DIPG-10. In a xenograft flank model, we observed increased quisinostat sensitivity in the KO models (20 day mean tumor volume of 547mm3 [parental], 396mm3 [PMS2 KO], and 150mm3 [MSH2 KO]). RNA sequencing of quisinostat-treated PBT-24 revealed an increase in acute inflammatory response genes which we are investigating as a mechanism of action for HDACi against hypermutant tumors. Ultimately, we demonstrate the in vivo efficacy of quisinostat against hypermutant DIPG that supports the investigation of HDAC inhibition as a treatment strategy across hypermutant CNS tumors.

DIPG-59. BLOOD BRAIN BARRIER (BBB) MODULATION USING CADHERIN PEPTIDES IN THE TREATMENT OF DIFFUSE MIDLINE GLIOMA OF THE PONS (DMG-P)

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BACKGROUND: Diffuse midline glioma of the Pons (DMG-P) is the most common brainstem tumor of childhood and is universally fatal. The BBB is frequently intact in DIPG and restricts entry of systemically administered conventional and biological therapies. We have synthesized and validated synthetic cadherin peptides (HAV6, HAVN1) that cause transient opening of the BBB by preventing the dimerization of the extracellular membrane bound VE-Cadherin in the vascular endothelium. METHODS: We used DMG-P tumor conditioned media (TCM) and co-cultures in two in-vitro model systems (trans well inserts, Blood Brain Tumor Barrier microfluidics model (BBTB-DMG-P microchip)-using Human Brain microvascular endothelial cells (HBMVECs) / SF8628 (DMG-P PDX) and validated the in-vitro findings in a DMG-P orthotopic murine model using RA055. The permeability of different molecular weight markers [(Gd-DTPA, IRDye 800CW PEG, rhodamine 800 (R800)] were examined in presence / absence of HAV6 / HAVN1 peptides. RESULTS: The BBTB integrity is maintained in-vitro as evidenced by decreased permeability of all the agents across the barriers in the TCM / co-culture models. Addition of HAV6/ HAVN1 peptides in-vitro increased the permeability of all the agents across the barriers. The permeability of R800 increased in the presence of Elacridar (P-gp inhibitor). The BBB was intact in the DMG-P murine model, as evidenced by minimal Gd-DTPA contrast enhancement in T1 weighted MRI images. Administration of HAV6 / HAVN1 peptides resulted in Gd-DTPA contrast enhancement of the tumor at 5- and 10-minutes post injection. CONCLUSION: Our data confirms that the BBTB is intact in DMG-P and can be transiently modulated using synthetic cadherin peptides. Transient BBTB opening using synthetic peptides will help BBB non-penetrant chemotherapy drugs like Vincristine/Mitoxantrone and biological therapies to reach intra-tumoral therapeutic levels and improve therapeutic efficacy.

DIPG-60. AVAPRITINIB FOR TARGETING PDGFRA IN H3K27M – MUTATED DIFFUSE MIDLINE GLIOMA

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H3K27M-mutated diffuse midline glioma (H3K27M DMG) are an almost universally fatal disease with a median survival of less than 6 months post progression and no effective therapy. PDGFRA-signaling has shown to promote and sustain a subset of oligodendrocyte precursor-like tumor cells that are responsible for tumor propagating potential and high proliferation rates. However, first attempts to target PDGFRA in adult glioblastoma with dasatinib/imatinib or pediatric refractory brain tumors with sunitinib were not successful. We report on the first experience in two patients receiving avapritinib, a highly potent, selective, brain penetrant PDGFRA/KIT inhibitor under a compassionate use program. Our first patient with spinal H3K27M DMG developed supratentorial metastases ten months after initial diagnosis. Molecular profiling revealed de novo PDGFRA and KIT amplifications and treatment with dasatinib was initiated. Due to disease progression and novel metastases, therapy was switched to avapritinib showing near complete resolution of the previously unirradiated frontal lesion with additional disease stabilization of other metastatic sites. Following re-resection and irradiation of progressing cerebellar lesions, the patient remains clinically stable on avapritinib therapy over 12 months. The second patient with diffuse intrinsic pontine glioma showed disease progression nine months after diagnosis and was treated with focal re-irradiation (30Gy). As the tumor harbored a PDGFRA R841del alteration, avapritinib was initiated seven weeks after radiation upon further tumor progression resulting in partial response. Pharmacokinetic sampling of cerebrospinal fluid (CSF) detected an increasing CSF/plasma ratio over time and up to 4 µM avapritinib in tumor tissue. Avapritinib CSF levels in both patients were distinctly higher than dasatinib levels. Avapritinib was generally well tolerated besides lower limb edema, elevated LDH and liver enzymes. Hence, effective CNS penetration of avapritinib at pharmacologically relevant brain tumor concentrations resulted in clinical response in two patients with rapidly progressive H3K27M DMG.

DIPG-61. PRECLINICAL EFFICACY OF COMBINED RADIOTHERAPY WITH VENETOCLAX TREATMENT IN TARGETING DIFFUSE MIDLINE GLIOMAS

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BACKGROUND: H3K27M diffuse midline gliomas (DMGs) are highly aggressive pediatric tumors of pons, thalamus or spinal cord. The only standard-of-care for DMGs is radiation therapy (RT) since the anatomical location of such tumors does not allow surgical resection. Tumor response to RT is at best transient as tumor becomes refractory due to radioresistance. Tumor relapse after RT is a major hurdle in treating DMGs. The mechanism of development of radioresistance due to RT-induced stress has not been studied in DMGs yet. METHODS: We performed an integrated genomic analysis to determine genes responsible for radioresistance and a targeted drug screen to identify drugs synergizing with radiation in DMG. Effect of venetoclax on radiation-naïve and 6Gy radiated DMG cells was evaluated by studying cell death and apoptosis. The efficacy of combining venetoclax with radiation was evaluated *in vivo* using orthotopic xenograft models. RESULTS: We identified that BCL2 as a key regulator of tumor growth in DMGs after radiation. Radiation sensitizes DMGs to venetoclax treatment. While venetoclax as a monotherapy was not cytotoxic to DMG cells, post-radiation venetoclax significantly increased cell death and apoptosis. Combining venetoclax with RT significantly enhanced the survival of mice with DMG tumors *in vivo*. Further, we found that the mechanism of radiation-induced cytotoxic effect of venetoclax is p53-independent in DMGs. CONCLUSIONS: This study shows that venetoclax impedes the anti-apoptotic function of radiation-induced BCL2 in DMG leading to apoptosis. Our results are encouraging because, in clinical settings, majority of the DMG patients, irrespective of the tumor p53 status, will benefit from combining RT with venetoclax treatment. Since venetoclax either alone or in combination with chemotherapy drugs are currently in clinical trials for other pediatric cancers, a phase 1b trial is imminent for treating DMGs with venetoclax in combination with radiation therapy.

DIPG-62. REDUCING THE LEVELS OF GENOMIC 5-HYDROXYMETHYLCYTOSINE BY INHIBITING THE TET PATHWAY INDUCES APOPTOSIS AND DECREASES PROLIFERATION IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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The H3K27M mutation, observed in 70%-90% of DIPG, causes global hypomethylation. The Ten-Eleven-Translocation enzymes, TETs, which convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) regulate the methylation patterns in the genome. Previously, we observed an increase in the 5hmC levels and altered TET activity in DIPG. We hypothesize that targeting the TETs in DIPG could restore the epigenetic balance. In this study, we target the TET pathway with cell-permeable 2-hydroxyglutarate (2HG) and Bobcat339, a cytosine-based TET inhibitor. We see a dose-dependent decrease in 5hmC (46-96% reduction) and increase in 5mC (~5fold increase) in multiple DIPG cell lines treated with 2HG. We see a corresponding increase in apoptosis and reduction in proliferation (~8fold increase in cleaved-Caspase3 signal (P=0.0001) and ~55% reduction in BrdU-incorporation measured by IF (P=0.0036) in JHH DIPG16A with similar results in JHH DIPG1, HSJD007, and SUDIPG6). We see a 54-90% reduction in 5hmC and ~3fold increase in 5mC with Bobcat 339 treatment in DIPG cell lines JHH DIPG1, JHH DIPG16A, HSJD007, and SUDIPG6. We also see a corresponding increase in apoptosis as measured by cleaved PARP western blot and decrease in proliferation as measured by BrdU incorporation (approximately 32-53% decrease in proliferation). Combined treatment with 2HG and Bobcat339 lead to greater decreases in 5hmC and increases in 5mC compared to single treatment, with synergistic suppression of cell growth as measured by SynergyFinder in HSJD007, JHH DIPG1, and JHH DIPG16A. In vivo studies targeting DIPG orthotopic xenografts are currently underway. These results suggest that TETs contribute to the hypomethylated state observed in DIPG. Thus, inhibiting the TETs can be explored as a potential therapeutic approach to treat DIPG.

DIPG-63. THERAPIES FOR DIFFUSE GLIOMAS IN PEDIATRIC PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Diffuse gliomas are one of the most challenging pediatric brain tumors of the current era. The new WHO classification has brought in a paradigm shift in the diagnosis and management of diffuse gliomas. Although these tumors are not surgically resectable, an integrated molecular analysis could make them more amenable to targeted agents. We conducted a thorough systematic review and meta-analysis of therapies for diffuse gliomas in pediatric patients. Methods: Using PRISMA guidelines, PubMed, (Medline), Cochrane, and Google Scholar database searches are being conducted using search terms "diffuse midline glioma", "diffuse midline astrocytoma" and "diffuse glioma management". PubMed search filters used to find relevant articles were "human" species, "English" language, and "age birth to 18 years". Results: Interim analysis using the PubMed database has revealed 387 articles of which 32 articles describe details of therapies used in pediatric patients. We found 8 case reports, 17 case series, and 7 clinical trials elucidating therapies for diffuse gliomas. Comprehensive analysis of modalities of therapies including neurosurgery, chemotherapy, and radiation therapy, along with survival analysis for these patients stratified by H3.3K27M mutant status is ongoing.

DIPG-64. FEASIBILITY AND EARLY RESULTS OF RADIOTHERAPY, CONCOMITANT NIMOTUZUMAB AND VINORELBINE AND RE-IRRADIATION AT PROGRESSION, FOR NEWLY DIAGNOSED CHILDHOOD AND ADOLESCENCE DIFFUSE MIDLINE GLIOMA (DMG)

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BACKGROUND: The purposes of this trial were evaluating the feasibility, response and PFS/OS of patients receiving upfront radiotherapy and reirradiation at progression with concomitant nimotuzumab/vinorelbine chemotherapy as the standard arm of the ongoing protocol for DIPG. METHODS: Patients aged 2-21 years with not-pretreated DMG (evidence of H3.3K27M mutation at immunohistochemistry) received vinorelbine 20 mg/m^2+nimotuzumab 150 mg/m^2 weekly for 12 weeks; thereafter every other week until tumor progression or for up to 2 years. Focal photons irradiation was delivered within the 3rd week after first nimotuzumab+vinorelbine administration with a total dose of 54 Gy in 1.8 Gy daily fractions. For local progression re-irradiation was proposed at 19.8 Gy; in case of dissemination craniospinal-irradiation at 36 Gy was adopted. MRI were blindly centrally reviewed at diagnosis and every 12 weeks thereafter. RESULTS: Aggregated preliminary results are available for 20 patients from 4 Italian centers: 12 males, median age 11 years (range 3-17). Median time of observation was 12.5 months; 8 had thalamic/basal ganglia disease, in 5 pons was involved (2 pontobulbar, 3 pontomesencephalic), 6 spinal, 2 cerebellar. 13 patients had only biopsy, the others partial or near-total resection.14 relapsed: 5 locally, 4 with dissemination, 5 with both; 12 died, one was lost at follow-up. Two patients received reirradiation, one of them was irradiated three times without evidence of radionecrosis, still alive at 26 months from diagnosis. Median EFS/OS were 8.3/10.2 months, respectively; EFS/OS at 1 year were 25.8/36.7%. Survival curves between spinal and cerebral locations showed no difference. Patients after partial/near-total resection vs biopsied seemed to have earlier relapses (P 0.017) with EFS at 6 months of 34.3/75.0% respectively. CONCLUSIONS: This is one of the first series of DMG homogeneously and prospectively treated; treatment was feasible. A potential role of reirradiation emerge as in DIPGs.

DRUG DELIVERY/PHARMACOKINETICS

DDEL-01. THE ROLE OF KEY PHARMACODYNAMIC AND PHARMACOKINETIC PARAMETERS IN DRUG RESPONSE PREDICTION OF PEDIATRIC TUMORS IN THE PRECISION ONCOLOGY STUDY INFORM

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INTRODUCTION: The INFORM (INdividualized Therapy FOr Relapsed Malignancies in Childhood) study is a European pediatric precision