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INTRODUCTION: DMG-ACT (DMG- multi-arm Adaptive and Combinatorial Trial) will implement an innovative clinical trial design of combinatorial arms for patients with DMG at all disease stages, that is adaptive to pre-clinical and correlate data generated in eight collaborating institutions. The goal of the team is to rapidly identify and validate i) promising drugs and drug combinations for clinical use, and ii) predictive biomarkers of promising drugs. METHODS: In vitro (n=30) and in vivo (n=8) models of DMG across fourteen institutions were used to assess single and combination treatment of over 80 drugs and drug combinations. Predictive biomarkers of response for top candidate drugs were identified using extensive molecular assays including proteomics, CRISPR, RNAseq, ELISA, FACS, and IHC. RESULTS: Inhibitory concentration (IC50) of all drugs were established and validated across all 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), ONC201+panobinostat (p=0.01). Marizomib was highly toxic in murine PDX and zebrafish larvae assays. Murine pharmacokinetic analysis showed peak brain levels of ONC201, and ONC206 above pre-clinical IC50 concentrations. Molecular testing and analyses of existing drug screen across 578 cancer cells validated mitochondrial stress and additional proteins, as the main targets induces by ONC201/6. CONCLUSION: Thorough preclinical testing in a multi-site laboratory setting identified promising therapeutics for DMGs, resulting in launch of two clinical trials (PNOC022, ONOC023). Validation of identified biomarkers are ongoing using clinical specimen as well as in vivo PDX models.

DIPG-50. BIOINFORMATIC EVALUATION OF GENES INVOLVED IN SPHINGOMYELIN BIOSYNTHESIS IN DIFFUSE MIDLINE GLIOMA H3K27 ALTERED/DIPG: DYSREGULATION OF SPHINGOSINE 1-PHOSPHATE (SP1)

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Sphingosine 1-phosphate (S1P), a bioactive signalling lipid, interacts with a network of metabolic enzymes, receptors, transporters, and epigenetic partners. This network is well described in many cancers; however, little is known about its potential impact in DIPG. Expression of HDAC1 (binding target of S1P) and genes associated with the sphingomyelin (SM) pathway were examined in datasets identified in the National Centre for Biotechnology Information, Gene Expression Omnibus, and analysed using the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl). The Paugh-DIPG dataset (27 DIPG samples) and normal samples (20 years and younger - Berchtold dataset) were compared. To avoid issues related to batch effects, expression values for each gene of interest and controls were exported into separate files to determine differentially expressed genes. Internal genes include housekeeping; ACTB, GAPDH, B2M, TBP; downregulated in DIPG; GPR6, NGB, and upregulated in DIPG; MMP16, PDGFRA, TP53, CSPG4. Genes of interest; SPHK1, SPHK2, SGPL1, ACER1, ACER3, KDSR, SMPD1-4, CPTP, GLTP, DEGS1, CERK, CERS1-6, ASAH1, SGPP1, SGPP2 and HDAC1. To test for significance, each dataset was standardised using ACTB housekeeping gene. Values including Log-transformed fold change were analysed using the non-parametric, Mann-Whitney test. 7 of the 16 genes were dysregulated relative to expression in normal brain ($p<0.0002$). SPHK2 and SMPD3 were downregulated, and HDAC1, SGPL1, DEGS1, CERS4, and ASAH1 were upregulated in DIPG compared to normal. To identify genes more likely associated with DIPG (vs development), we evaluated gene expression in Brainspan dataset (brspv10rs). Validation of SPHK2 and SGPL1 protein expression (responsible for the synthesis and cleavage of SP1) is underway. Current work is focused on the intracellular processing and function (isoform specific inhibitors) of S1P in DIPG cells. Given its reported role in several cancer hallmarks, a better understanding of the sphingomyelin biosynthesis pathway in DMG/DIPG is merited and may lead to novel therapeutic targets.

DIPG-51. HYDROCEPHALUS TREATMENT AND THE EFFECT ON SURVIVAL IN DIFFUSE INTRINSIC PONTINE GLIOMA

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BACKGROUND: Diffuse intrinsic pontine glioma (DIPG), can cause hydrocephalus and if symptomatic, leads to rapid changes in consciousness requiring surgical intervention. The effect of cerebrospinal fluid (CSF) diversion on overall survival and the clinical factors influencing outcome remain unclear. The aim of this study was to evaluate the impact of the treatment of hydrocephalus on survival in DIPG patients. METHODS: The study was retrospective in design using data from the SIOPE-European Society of Pediatric Oncology DIPG Registry. Hydrocephalus was determined based on a centrally reviewed diagnostic MRI. The Kaplan-Meier method was used for survival statistics. Clinical prognostic factors including: duration of symptoms, age and cranial nerve palsy at diagnosis were evaluated for confounding and effect modification. The effect of hydrocephalus treatment (CSF diversion) on survival was examined using Cox regression. RESULTS: Among 582 patients from the SIOPE-DIPG Registry, 86 (14%) had hydrocephalus at diagnosis. Median OS for hydrocephalus patients treated with CSF diversion (n=43) was 13 months (95% CI, 10.2-17.7) and 9 months (95% CI, 7.4-10.6) for hydrocephalus patients without a CSF diversion (n=43). Survival rates were not significantly different ($p=.41$). On adjusted Cox regression, correcting for duration of symptoms, hydrocephalus patients with signs of cranial nerve palsy at diagnosis and a CSF diversion had a hazard ratio 0.476 ($p=0.004$). CONCLUSION: Survival among DIPG patients presenting with hydrocephalus at diagnosis was not influenced by CSF diversion. Hydrocephalus patients with signs of cranial nerve palsy at diagnosis, had a significantly reduced risk after undergoing CSF diversion. There is an indication this subgroup of DIPG patients may benefit more from CSF diversion, although the relationship between hydrocephalus and cranial nerve palsy requires further investigation.

DIPG-52. ACTIVATORS OF THE INTEGRATED STRESS RESPONSE SYNERGIZE TO KILL DIPG

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DIPG has elevated baseline activation of the integrated stress response (ISR), an evolutionarily conserved system that allows cells to tolerate various forms of stress. Increased expression of activating transcription factor 4 (ATF4) indicates activation of the ISR. Intermediate levels of ATF4 protect cells from stress, while sustained high levels result in cell death. The imipridone drug ONC201 binds to and activates the mitochondrial protease ClpP, leading to increased mitochondrial stress and persistent ATF4 activation. Because DIPG has a high baseline level of ATF4, we hypothesized that the ISR activators Sal003, ONC201, and fenretinide would synergize to kill DIPG. Sal003 inhibits dephosphorylation of ATF4 upstream regulator, eIF2α. The retinoic acid derivative fenretinide induces ATF4, increases reactive oxygen species, and has clinical activity in pediatric patients with neuroblastoma. After determining the IC25 of Sal003, fenretinide, and ONC201, we treated patient-derived DIPG cell lines with low micromolar doses. The combination of Sal003 and ONC201 significantly increased apoptosis as measured by CC3 immunofluorescence in comparison to DMSO ($p<0.0001$, ANOVA). Combination therapy also significantly increased CC3 positivity compared to single treatment. Western blots for cleaved PARP expression detected induction of apoptosis in DIPG treated with both Sal003 and ONC201 over DMSO and monotherapy treated cells. In some cell lines, the combination increased ATF4 expression. Since Sal003 is not yet available for clinical testing in humans, we treated DIPG cells with ONC201 and fenretinide. CC3 immunofluorescence indicated synergistically elevated apoptosis in the combination of ONC201 and fenretinide vs. DMSO ($p<0.0001$, ANOVA). Western blots showed increased cleaved PARP, ATF4, and CHOP expression in DIPG treated with ONC201 and fenretinide. We are currently testing the efficacy of this combination in orthotopic DIPG xenografts. Our results suggest the combination of ONC201 with fenretinide could potentially serve as a therapy for DIPG.

DIPG-53. LONG-TERM SURVIVAL FROM A PHASE 1 DOSE-ESCALATION TRIAL USING CONVECTION-ENHANCED DELIVERY (CED) OF RADIOIMMUNOTHERAPEUTIC ^{124}I-OMBURTAMAB FOR TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG).

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BACKGROUND: Median survival from DIPG is less than one year. In a phase 1 dose escalation study (clinicaltrials.gov NCT01502917) ^{124}I-omburtamab targeting B7-H3 was administered intratumorally using

CED. METHODS: CED was performed between 4-14 weeks post radiation therapy. Using a 3 + 3 design, ^{124}I-omburtamab was escalated from 0.25-10.0 mCi and infusion volumes (Vi) from 250-10,000 µl with serial ^{124}I PET/CT performed up to ~1 week post-administration. Toxicities were assessed for 30 days. Dose escalation safety was evaluated. Survival was calculated using Kaplan-Meier statistics. RESULTS: 46 children were treated and evaluable for toxicity and survival;4 patients received partial doses and were evaluable for toxicity only. Three patients experienced dose limiting toxicities. Eleven patients had transient treatment related grade 3 toxicities with no grade 4 or 5 toxicities. Grade 3 nervous system toxicities included: muscular weakness(n=8), dysarthria(n=4), ataxia(n=3), dysphagia(n=3), and gait disturbance(n=1). Lesion absorbed doses ranged from 1,000-1,500cGy/mCi, with lesion-to-whole body radiation absorbed-dose ratios of ~900. A dose of 8mCi and infusion volume of 8,000 µl is safe and may provide a distribution volume up to 20cm^3. Median survival was 15.3 months (n =46, 95% CI 12.7, 17.3). Survival rate estimates (95% CI) at 1, 2, 3 and 5 years were 0.67 (0.55;0.82); 0.18 (0.09;0.35); 0.10 (0.04;0.26); and 0.05 (0.01;0.20). Four patients survived >3 years; two remain alive (61+ and 106+ months);two have died (44 and 53 month) with distant CNS disease and one with extra-CNS metastasis. **CONCLUSION:** Administration of escalating doses and volumes of ^{124}I-omburtamab via CED was a viable option for this patient subgroup. The median overall survival was increased 3-4 months compared to historical controls. Anecdotal long-term survival if validated with a planned phase 2 trial would support the concept of whole neuroaxis treatment in combination with CED in a subset of DIPG patients.

DIPG-54. P53 PATHWAY REACTIVATION AS A THERAPEUTIC STRATEGY IN DIFFUSE INTRINSIC PONTINE GLIOMA

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TP53 is the most frequently mutated tumor suppressor with somatic alterations found in approximately 50% of all human cancers. In the remaining TP53 wild-type (WT) tumors, functional inactivation of the p53 pathway may be achieved through a variety of other mechanisms, including gene deletion, epigenetic silencing, and/or alterations in prominent negative regulators, including MDM2/MDM4 and PPM1D. These alterations block p53 activity and lead to uncontrolled cell proliferation and oncogenesis in a majority of cancers, including the highly aggressive, universally fatal glial cell tumors of childhood, known as Diffuse Intrinsic Pontine Gliomas (DIPGs). DIPGs are inoperable due to their location within the brainstem. Available treatment options, including radiotherapy, have had a palliative effect at best, with almost all children succumbing to the disease within 18 months of diagnosis. Recent advances have led to an improved understanding of the biological underpinnings of this disease and identification of recurrent genetic alterations that represent potential therapeutic targets for these patients. Prominent among these targets in DIPGs with WT p53 status (50%) are MDM2/4 and PPM1D, whose suppression lead to p53 reactivation specifically in the WT p53 context. We have undertaken a combination of approaches to better understand the therapeutic potential of MDM2 and PPM1D inhibition in DIPG, characterizing the genomic, transcriptomic, and cell-state changes that drive resistance, and identifying novel vulnerabilities that can be exploited with combination therapies towards a cure.

DIPG-55. INCREASING THE DRUG-TUMOR RESONANCE TIME IN DMG MURINE MODELS SIGNIFICANTLY EXTENDS SURVIVAL

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H3K27-altered diffuse midline glioma (DMG) is a uniformly lethal CNS cancer that predominately occurs in children. In the last decade, while studies revealing the molecular underpinnings of the disease have paved the way for novel therapeutic strategies, this tumor remains a death sentence. Our lab and others have identified numerous drugs of high interest for treating this devasting disease, however, every trial has failed to show benefit. Is our failure to translate these laboratory findings due to the fact we have not found the right drugs or treatment regimens yet? Or is this a failure to get the drugs to the tumor? Or is this simply a very bad disease to treat? The answer is likely multifactorial. Our lab, in collaboration with others, have strong data to show that simply getting drugs to the tumor will not result in a positive benefit. Most drugs are rapidly cleared from their target space. Utilizing techniques that increase the drug-tumor resonance time is necessary to translate our basic science findings to our patients. Picking the right drug and the delivery mechanism is not enough for a positive effect. Here, we assessed the efficacy of a previously identified inhibitor, alisertib, delivered by short-term convection-enhanced delivery or a continuous osmotic pump and found that continuous, direct delivery results in a significant increase in survival (p = 0.0002). Furthermore, long-term survivors showed a decreased tumor burden and on-target drug effects. These data support that translation into patients must consider three factors: 1) selection of a potent therapy, 2) selection of the proper delivery method for that therapy, 3) modulation to ensure that the therapy remains in the targeted tissue long enough for desired effect. Keeping these basic tenets at the forefront of development may finally lead to a more effective treatment strategy for this devastating disease

DIPG-56. DEVELOPMENT AND APPLICATION OF A NOVEL ANTIBODY AGAINST CD99 AS A THERAPEUTIC STRATEGY IN DIFFUSE MIDLINE GLIOMA

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BACKGROUND: There is an unmet need to identify novel targeted therapies for Diffuse Midline glioma (DMG) which is currently a refractory disease. Recently, we identified high expression of a cell surface antigen, CD99 in H3K27M-mutant expressing DMGs compared to other normal brain counterparts. We developed a novel chimeric CD99 antibody and tested the anti-tumor efficacy of this antibody in vitro and in vivo. **METHOD:** Bio-legend cell-surface screening was performed in H3K27M-mutant and WT DMG cells. Functional role of CD99 was studied using CD99 proficient and depleted tumors. Designed and synthesized CD99 antibody with a new binding sequence on a human IgG scaffold and performed cell toxicity and growth-inhibitory studies using DMG tumor and normal cells. We also performed these studies in combination with radiation. Multiple patient-derived orthotopic DMG xenograft models was used to test the antibody efficacy. Different antibody delivery routes, that are clinically relevant were investigated. RESULTS: CD99 expression is transcriptionally regulated by H3K27M and is enriched on the cell surface of K27M tumors compared to WT DMG tumors. Our new CD99 antibody (10D1 clone) significantly reduced DIPG tumor cell proliferation in vitro. Intravenous infusion of this antibody in DIPG tumor bearing mice showed complete clearance of tumor that prolonged animal survival suggesting the enhanced anti-tumor efficacy of 10D1-CD99 and importantly, its ability in crossing the blood-brain-barrier and reaching the pons target site. Loco-regional administration of 10D1 showed similar anti-tumor effects even at much reduced antibody concentrations while toxicity to CD99-expressing T cells was minimum. Radiation increased CD99 expression and enhanced the cytotoxic effect of 10D1-CD99. **CONCLUSION:** We have developed a novel CNS penetrant CD99 antibody that is an attractive therapeutic strategy in treating DMG. 10D1 is currently in development as a therapeutic.

DIPG-57. A SYSTEMS BIOLOGY APPROACH TO DEFINING AND TARGETING MASTER REGULATOR DEPENDENCIES FROM BULK AND SINGLE-CELL RNA-SEQ IN DIFFUSE MIDLINE GLIOMA (DMG)

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Diffuse midline glioma (DMG) are fatal pediatric brain tumors with no effective systemic therapies. Molecular profiling demonstrates epigenetic dysregulation and heterogeneity, and novel approaches are needed to identify promising drugs and drug combinations. We used network-based computational analysis of RNA-seq to discover Master Regulator (MR) proteins that represent targetable, mechanistic determinants of distinct DMG cell states. We reverse-engineered the first DMG-specific regulatory network from 122 publicly available DMG RNA-seq profiles with ARACNe. Using this network, we measured sample-specific protein activity based on differential expression of their targets via VIPER. Activity-based clustering identified two clusters showing a trend in survival differences (>1 year, by $\chi 2$). The most activated MRs (i.e., TOP2A, CENPF, BUB1B, FOXM1, GTSE1, MKI67, E2F8), relative