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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