RT response, RT treated tumors have increase in cell cycle regulatory genes such as Cdkn1a, across all clusters. In non-resident myeloid cells, compared to untreated tumor, RT is associated with a robust upregulation of interferon response genes in both macrophages (Isg15 Fold Change (FC) 2.30; Ifit1 FC 1.64; Ifit3 FC 2.02; Cxcl10 FC 2.29) and dendritic cells (Isg15 FC 2.67; Ifit1 FC 1.72; Ifit3 FC 2.06; Cxcl10 FC 1.50). We also find differential expression of immune checkpoints in RT-treated versus untreated tumor with decreased expression of Lag3, Tim3 (Havcr2), and Csf1R and increased expression of Cd47, Sirpa and Gitr (Tnfrsf18) post RT. In summary, RT stimulates a pro-inflammatory TIME response and alters immune checkpoints in DMG, highlighting the potential for combining RT and immunotherapy in these tumors.

DIPG-46. RADIATION INDUCED SENESCENCE IN DIFFUSE INTRINSIC PONTINE GLIOMA CELLS REVEALS SELECTIVE VULNERABILITY TO BCL-XL INHIBITION

Ashley Vardon[1], Romain Guiho[1], Diana Carvalho[2], Jessica Boult[2], Rebecca Carter[1], Yura Grabovska[2], Alan Mackay[2], Guangrong Zheng[3], Daohong Zhou[3], Crispin Hiley[1], Mark Lythgoe[1], Chris Jones[2], Darren Hargrave[1], Juan-Pedro Martinez-[1]; [1]University College London, London, United Kingdom. [2]Institute of cancer research, London, United Kingdom. [3]University of Florida, Florida, USA

Diffuse intrinsic pontine glioma remains a devastating condition with a dismal five year survival rate less than 5%. New approaches for treating this aggressive disease are critical to driving progress. Conventional radiotherapy remains the cornerstone of treatment, with no chemotherapeutic agent found to improve survival. However, radiotherapy is often delivered as a palliative treatment, and disease often recurs 3-6 months after. Radiation causes DNA damage and oxidative stress yielding a senescent state of replicative arrest in susceptible cells. However, increasing evidence demonstrates malignant cells can escape senescence leading to tumour recurrence. Targeted ablation of non-replicating senescent tumour cells following radiation could negate tumour recurrence. It remains unknown whether DIPG undergoes senescence following radiation, and furthermore, whether senolytics can be utilised to target senescent DIPG cells. We employed radiation to induce a senescent state in primary human DIPG cell lines. Senescence was confirmed using SA-β-gal staining, lack of EdU incorporation and qRT-PCR to characterise the SASP in three primary human DIPG cell lines. RNA-sequencing on DIPG cells following radiation revealed senescence and SASP signatures. Likewise, expression of senescence markers has been detected in human tumours. Viable cells that survive radiation were then utilised to screen candidate senolytic drugs, only Bcl-XL inhibitors demonstrated reproducible senolytic activity in radiation treated DIPG cells. In addition, Bcl-XL degradation using PROTACs (proteolysis targeting chimeras) resulted in a significant increase in senolysis of susceptible tumour cells. Conversely, Bcl-2 inhibitors failed to show any consistent senolytic activity. We are currently performing preclinical studies in the mouse to test the efficiency of senolytics against DIPG. These results demonstrate future possibilities of targeting radiation induced senescence in DIPG, using novel senolytic therapies and highlight Bcl-XL dependency as a potential vulnerability of surviving DIPG cells following exposure to radiation.

DIPG-47. TSO500CTDNA SEQUENCING REVEALS ONCOGENIC MUTATIONS AND COPY NUMBER VARIATIONS IN THE LIQUID BIOME OF CHILDREN WITH DIFFUSE MIDLINE GLIOMA

Erin R. Bonner[1,2], Robin Harrington[3], Augustine Eze[1], Miriam Bornhorst[4], Cassie N. Kline[5], Adam Dawood[1], Biswajit Das[3], Li Chen[3], Rini Pauly[3], P. Mickey Williams[3], Chris Karlovich[3], Amanda Peach[3], D'Andra Howell[3], James Doroshow[6], Lindsay Kilburn[4], Roger J. Packer[4], Sabine Mueller[7,8], Javad Nazarian[1,8]; [1]Center for Genetic Medicine Research, Children's National Hospital, Washington, DC, USA. [2]The George Washington University, Washington, DC, USA. [3]Molecular Characterization Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD, USA. [4]Children's National Hospital, Washington, DC, USA. [5]Children's Hospital of Philadelphia, Philadelphia, PA, USA. [6]Developmental Therapeutics Clinic/Early Clinical Trials Development Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD, USA. [7]Department of Neurology, Neurosurgery and Pediatrics, University of California San Francisco, San Francisco, CA, USA. [8]University Children's Hospital Zurich, Zurich, Switzerland

BACKGROUND: Molecular profiling of childhood CNS tumors is critical for diagnosis and clinical management, yet tissue access is restricted due to sensitive neuroanatomical locations. Moreover, CNS tumors including diffuse midline glioma (DMG) exhibit mutational heterogeneity and clonal evolution, which cannot be captured by upfront diagnostic biopsy alone. To address the lack of tumor visibility, and tprovide opportunity for longitudinal sampling, we validated and optimized a commercially available deep sequencing platform for analysis of circulating tumor DNA (TSO500ctDNA™). METHODS: In a proof-of-concept study, we defined the sensitivity, specificity, and clinical relevance of our novel ctDNA platform via analysis of paired tissue, CSF, and blood from children with DMG (n=10). Paired samples were assessed for concordance and sequencing results were compared to digital droplet PCR (ddPCR) detection of prognostic H3K27M mutation. RESULTS: DMG associated mutations in genes including *H3-3A, H3C2, TP53*, and *ACVR1* were detected in ctDNA, including in CSF samples with low (<5ng) starting DNA input. Of 9 H3K27M mutations identified in tumor, 8 were present in CSF and 3 in plasma/serum, for a positive percent agreement with tumor results of 89% and 33%, respectively. Among CSF samples, H3.3K27M was detected in 6/6 cases, and H3.1K27M in 2/3 cases, with variant allele frequencies comparable to ddPCR results. CNVs including *PDGFRA, KIT*, and *MDM4* gains were detected in CSF and paired tumor. Low frequency events including ACVR1, PIK3CA activating mutations and *KRAS* amplification were detected in CSF but absent from paired tumor, indicating tissue heterogeneity. Strategies to optimize ctDNA detection, including optimization of ctDNA isolation and adjustment of library QC metrics, were identified. **CONCLUSION**: Targeted ctDNA deep sequencing is feasible, can inform on clinically relevant tumor mutation and CNV profiling, and provides an opportunity for longitudinal monitoring of tumor genomic evolution in the liquid biome of children with CNS tumors.

DIPG-48. MRI VOLUMETRIC AND MACHINE LEARNING BASED ANALYSES PREDICT SURVIVAL OUTCOME IN PEDIATRIC DIFFUSE MIDLINE GLIOMA

Erin R. Bonner[1,2], Xinyang Liu[3], Carlos Tor-Diez[3], Madhuri Kambhampati[1], Augustine Eze[1], Roger J. Packer[4], Javad Nazarian[1,5], Marius George Linguraru[3], Miriam Bornhorst[1,6]; [1]Center for Genetic Medicine Research, Children's National Hospital, Washington, DC, USA. [2]The George Washington University, Washington, DC, USA. [3]Sheikh Zayed Institute for Pediatric Surgical Innovation, Children's National Hospital, Washington, DC, USA. [4]Children's National Hospital, Washington, DC, USA. [5]University Children's Hospital Zurich, Zurich, Switzerland. [6]Gilbert Family Neurofibromatosis Institute, Children's National Hospital, Washington, DC, USA

INTRODUCTION: Diffuse midline glioma (DMG) is a fatal childhood CNS tumor. Magnetic resonance imaging (MRI) is the gold standard for DMG diagnosis and monitoring of response to therapy. Leveraging novel MRI analytical approaches, including volumetric and machine learning based analyses, may aid in the prediction of patient overall survival (OS) and help to identify high-risk cases. METHODS: T1- and T2-weighted MR images were retrospectively collected from children and young adults diagnosed with DMG (n=43). MRI features, including manually determined 3D tumor volume (T2), T1 contrast-enhancing tumor volume, T1 relative to T2 volume (T1/T2), tumor relative to whole brain volume, tumor average intensity, and tumor heterogeneity (i.e., intensity skewness and kurtosis), were evaluated at upfront diagnosis. MRI features were analyzed to identify significant predictors of OS outcome, which was defined as OS shorter, or longer, than one year from diagnosis. A support vector machine was used to predict OS outcomes using combinations of these features. RESULTS: The presence of T1 contrast-enhancing tumor at diagnosis (p=0.01), and a high T1/T2 ratio (>25%, p=0.009), predicted significantly shorter median OS. Moreover, feature selection identified T2 mean intensity (p<0.001), T2 image intensity skew (p=0.006), T1/T2 ratio (p=0.02), and T1 volume relative to whole brain (p=0.03) as significant predictors of OS outcome (short versus long). Combining T2 mean intensity, T2 image skew, T1 segment kurtosis and patient gender resulted in OS outcome prediction accuracy of 83.3% (sensitivity=85%, specificity=81.8%, n=42 cases).CONCLUSION: We have identified MRI volume and imaging features that significantly predict OS outcome in children diagnosed with DMG. Our findings provide a framework for incorporating MRI volumetric and machine learning analyses into the clinical setting, allowing for the customization of treatment based on tumor risk characteristics.

DIPG-49. INTERNATIONAL PRECLINICAL DRUG DISCOVERY AND BIOMARKER PROGRAM INFORMING AN ADOPTIVE COMBINATORIAL TRIAL FOR DMG

Javad Nazarian[1,2], Matthew Dun[3], Lindsay Kilburn[2,4], Sebastian Waszak[5,6], Nicholas Vitanza[7,8], Andrea Franson[9,10], Mike Prados[11,6], Eric Raabe[12,13], Ron Firestein[14,15], Alexander Beck[16], Amanda Saratsis[17], Barak Rotblat[18], Dannis van Vuurder[19,20], Jessica Foster[21], Esther Hulleman[19], Cassie Kline[21,22], Nalin Gupta[11,6], Jason Cain[14,15], Carl Koschmann[9,10], Sabine Muller[11,1]; [1]University Children's Hospital Zurich, Zurich, Switzerland. [2]Children's National Hospital, Washington, DC, USA. [3]University of Newcastle, Callaghan, NSW, Australia. [4]George Washington University, Washington, DC, USA. [5]University of Oslo, Oslo, Norway. [6]University of California, San Francisco, San Francisco, California, USA. [7]Seattle Children's Hospital, Seattle, Washington, USA. [8]University of Washington School of Medicine, Seattle, Washington, USA. [9]C.S. Mott Children's Hospital, Ann Arbor, Michigan, USA. [10]University of Michigan,

Ann Arbor, Michigan, USA. [11]UCSF Benioff Children's Hospitals, San Francisco, California, USA. [12]Johns Hopkins Hospital, Baltimore, Maryland, USA. [13]Johns Hopkins University, Baltimore, Maryland, USA. [14]Hudson Institute of Medical Research, Melbourne, Victoria, Australia. [15]Monash University, Melbourne, Victoria, Australia. [16]Ludwig Maximilian University of Munich, Munich, Germany. [17]Lurie Children's Hospital of Chicago, Chicago, Illinois, USA. [18]Ben Gurion University of the Negev, Be'er Sheva, Israel. [19]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands. [20]VU University Medical Center, Amsterdam, Netherlands. [21]Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA. [22]University of Pennsylvania, Philadelphia, Pennsylvania, USA

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)

Aimee Humphreys, Hanna Russell, Helen L Fillmore; University of Portsmouth, Portsmouth, United Kingdom

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

Joshua Baugh[1], Nada Ben Mohammed[1,2], Sophie Veldhuijzen van Zanten[1,3], Christof Kramm[4], Veronica Biassoni[5], Maura Massimino[5], Dannis van Vuurden[1]; [1]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands. [2]Amsterdam UMC, Vrije Universiteit Faculty of Medicine, Amsterdam, Netherlands. [3]Erasmus University Medical Center, Department of Radiology and Nuclear Medicine, Rotterdam, Netherlands. [4]Division of Pediatric Hematology and Oncology, University Medical Center Goettingen, Goettingen, Germany. [5]Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale dei Tumori, Milan, Italy

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

Orlandi Novak, Antje Arnold, Charles Eberhart, Eric Raabe; Johns Hopkins University School of Medicine, Baltimore, MD, USA

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).

Mark M. Souweidane[1,2], Kim Kramer[1], Neeta Pandit-Tasker[1], Sofia Haque[1], Pat Zanzonico[1], Jorge Carrasquillo[1], Serge K. Lyashchenko[1], Sunitha B. Thakur[1], Yasmin Khakoo[1], Ira J. Dunkel[1], Maria Donzelli[1], Jason S. Lewis[1], Nai-Kong V. Cheung[1], Steve M. Larson[1], Anne S. Reiner[1], Katherine S. Panageas[1], Nicole Manino[1], John Rømer Nielsen[3]; [1]Memorial Sloan Kettering Cancer Center, New York, NY, USA. [2]New York-Presbyterian/Weill Cornell Medical Center, New York, NY, USA. [3]Y-mAbs Therapeutics, Inc., Horsholm, Denmark

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