findings in the mouse brain, EZHIP is not expressed at any time point or spatial location within the developing human brain. Taken together, these data reject the above hypothesis, that EZHIP or H3K27M co-opt an endogenous PRC2 inhibitory developmental program. Similarly, these results show that EZHIP is not expressed within the cell of origin for PFA or DMG. Further studies will seek to understand the endogenous function of EZHIP by further defining its normal expression pattern and function.

TBIO-10. NGS MOLECULAR PROFILE OF PAEDIATRIC BRAIN TUMOURS: RESULTS FROM 92 CONSECUTIVE PATIENTS TREATED AT CENTRO HOSPITALAR UNIVERSITÁRIO DE SÃO JOÃO

Jorge Lima[1,2], Jorge Pinheiro[3], Susana Nunes[3], Ana Paula Fernandes[3], Paula Soares[1], Jose Carlos Machado[1,2], Josue Pereira[3], Maria Joao Gil da Costa[3]; [1]i3S, Porto, Portugal. [2]Ipatimup, Porto, Portugal. [3]Centro Hospitalar Universitario de Sao Joao, Porto, Portugal

AIM: Our aim was to progress in bringing molecular medicine to routine clinical practice in the setting of paediatric neuro-oncology. We have implemented a protocol between Ipatimup and Centro Hospitalar Universitário de São João for the rapid and efficient delivery of the molecular portrait of paediatric brain tumours. MATERIAL AND METHODS: We have enrolled 92 patients with the following inclusion criteria: Age 0-18 years; newly diagnosed brain tumour; previously diagnosed brain tumour, whenever it presented as rare, aggressive or refractory disease; availability of tumour material; signed informed consent. Tumour samples were centrally reviewed by expert pathologists and profiled using the Oncomine Childhood Cancer Research Assay. RESULTS: In the 92 tumours that were molecularly profiled, *BRAF* was the most frequently altered gene, especially in pilocytic astrocytomas, being also detected in other LGG and HGG. Other commonly mutated genes were *PIK3CA* and *FGFR*, the former in HGG and the latter in LGG. *MYB* and *RAF1* rearrangements were also found in low grade glial/glioneuronal tumours, while HGG showed a more complex profile, with many cases harbouring multiple alterations in *EGFR, PDGFRA, ATRX, H3F3A, HIST1H3B, TP53*, among others. A 16-year old patient with CMMR (homozygous mutation in *PMS2*) developed a glioblastoma that carried nearly 5x the average number of mutations. Among the 8 medulloblastomas, 2 showed mutations in the SHH pathway (1 in *PTCH1* and one in *SUFU*) and 2 in the WNT pathway (1 in *CTNNB1* and one in *APC*). In the remaining cases, one ependymoma presented *MYCN* amplification, while no alterations were detected in 3 patients. CONCLUSIONS: This study enabled the detailed molecular study of 92 paediatric brain patients, allowing a more robust tumour classification and the identification of actionable alterations. A subset of the patients are already undergoing targeted therapy, mainly using BRAF or MEK inhibitors with generally good improvement.

TBIO-11. THE GLUTAMINE TRANSPORTER AND CANDIDATE DIAGNOSTIC AND THERAPEUTIC TARGET SLC1A5 IS ASSOCIATED WITH SUBTYPE-SPECIFIC METABOLIC PHENOTYPES AND TUMOR PROGNOSIS IN PEDIATRIC BRAIN CANCERS

Adam Kraya[1], Run Jin[1], Chao Zhao[2], Ariana Familiar[1], Kathryn Wellen[3], Adam Resnick[1], Ali Nabavizadeh[1,4]; [1]Center for Data-Driven Discovery in Biomedicine, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [2]Department of Biomedical and Health Informatics, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [3]Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA, USA. [4]Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA

Glutamine transporters play an important role in supporting increased tumor nutritional demands relative to non-cancerous cells, often through overexpression of the solute carrier (SLC) family of membrane transporters. Preclinical studies in adult cancers demonstrate that targeting glutamine addiction via SLC1A5 inhibition results in growth-inhibitory and tumoricidal effects. Given their relatively higher expression in cancer versus normal brain tissue, SLC transporters represent compelling targets for molecularly-targeted radiation and application of available prognostic amino-acid PET imaging probes. However, the role of SLC transporters in pediatric brain cancers has yet to be investigated. We aimed to understand the relationship of SLC transporter expression with pediatric brain tumor subtypes and their potential prognostic significance using data from the Pediatric Brain Tumor Atlas (PBTA). Using the expression of amino acid transporter genes in ensemble survival models (Reactome: R-HSA-352230), we found that elevated expression of glutamine transporters (SLC1A5, SLC7A5, SLC7A11, SLC38A5, SLC38A3) predicted shorter progression-free survival (PFS) in low-grade gliomas (LGGs) and poorer overall survival in pediatric ependymomas, high-grade gliomas (HGGs), and medulloblastomas. We focused specifically on SLC1A5 given the availability of imaging probes (18 F-Fluoroglutamine and 18F-Fluciclovine) for the corresponding amino acid transporter (ASCT2). Through transcriptome-based consensus clustering, we found that supratentorial, RELA fusion-positive ependymomas and sonic hedgehog-activated medulloblastomas were over-represented among clusters expressing higher levels of SLC1A5 ($p = 3.38e-7$ and $p = 2.18-26$, respectively). Kaplan-Meier analysis found that higher expression of SLC1A5 was associated with shorter OS in ependymoma and medulloblastoma ($p = 9.8e-4$ and $p = 0.032$) and shorter PFS in LGG ($p = 0.022$). Gene set analysis showed higher expression and network rewiring of amino acid, lipid, and immune pathways in SLC1A5-high expressing clusters. Our work demonstrates that glutamine transporters, particularly SLC1A5, represent compelling targets in pediatric brain cancers that warrant further investigation for molecularly-targeted treatment and amino-acid PET imaging.

TBIO-12. SCREENING FOR CANCER PREDISPOSITION SYNDROMES IN PEDIATRIC NEURO-ONCOLOGY PATIENTS: A SINGLE INSTITUTION EXPERIENCE

Claire Johns[1], Cassie Kline[2], David Solomon[1], Sabine Mueller[1], Anu Banerjee[1], Nalin Gupta[1], Alyssa Reddy[1]; [1]University of California, San Francisco, San Francisco, CA, USA. [2]The Children's Hospital of Philadelphia, Philadelphia, PA, USA

BACKGROUND: Traditional screening for cancer predisposition syndromes centers on family history, phenotypic features, and tumor histology. With expanded accessibility of next generation sequencing, identification of de novo germline mutations is increasing and the predictive value of family history has become less clear. While identification of germline mutations often affects care of pediatric neuro-oncology patients, germline sequencing is not currently standard of care. We hypothesize that routine screening for germline mutations in pediatric neuro-oncology patients reveals unsuspected cancer predisposition syndromes and impacts care. METHODS: A retrospective analysis was performed on pediatric neuro-oncology patients at a single institution who had targeted next generation sequencing of approximately 500-cancer associated genes (UCSF500) on tumor and paired germline DNA. We determined the proportion of patients with germline mutations and assessed impact on future screening and current tumor treatment. We interrogated clinical notes, family history, and interviewed treating physicians to determine if predispositions were previously suspected. RESULTS: Between June 2015-December 2019, 187 patients had paired testing; of these 29 (16%) harbored germlines mutations that were pathogenic or likely pathogenic. Germline mutations were found in patients with high-grade glioma (n=12), low-grade glioma (n=7), medulloblastoma (n=4), ATRT (n=2), and choroid plexus papilloma (n=1). Known cancer predisposition syndromes were identified or confirmed in 18 patients. Of these, the most common alterations were in TP53 (n=6), CHEK2 (2), NF1 (n=2), SMARCB1 (n=2), and PTEN (n=2). Patients were referred to genetic counseling in 26 cases and malignancy screenings were implemented in 25 cases. Germline findings affected malignancy treatment in 10 cases, most often through use of targeted therapeutics or avoidance of radiation. CONCLUSIONS: In our series, we found that 16% of pediatric neuro-oncology patients harbored germline mutations, the majority of which were associated with cancer predisposition syndromes. These results support standardizing screening for pathogenic germline mutations in pediatric neuro-oncology patients.

VIRAL/GENE THERAPY AND OTHER NOVEL THERAPIES

THER-01. PRECISION BRAIN TUMOR THERAPY BY AAV-MEDIATED ONCOGENE EDITING

Laura von Soosten[1,2], Janina Haar[3], Veronika Frehtman[4], Stefan Holderbach[3,5], Julius Upmeier zu Belzen[5,6], Michael Jendrusch[5,7], Konstantin Okonechnikov[8], Stefan M. Pfister[8,9], Dirk Grimm[3,10], Barbara Leuchs[4], David Jones[1], Lena M. Kutscher[11], Marc Zuckermann[1,8]; [1]Division of Pediatric Glioma Research, Hopp Children's Cancer Center Heidelberg (KiTZ) and German Cancer Research Center (DKFZ), Heidelberg, Germany. [2]Faculty of Biosciences, Heidelberg University, Heidelberg, Germany. [3]Department of Infectious Diseases/Virology, Medical Faculty, University of Heidelberg, BioQuant, Heidelberg, Germany. [4]Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [5]Synthetic Biology Group, BioQuant Center, University of Heidelberg, Heidelberg, Germany. [6]Digital Health Center, Berlin Institute of Health (BIH) and Charité University Medicine, Berlin, Germany. [7]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. [8]Division of Pediatric Neurooncology, Hopp Children's Cancer Center Heidelberg (KiTZ) and German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany. [9]Department of Pediatric Oncology, Hematology, Immunology and Pulmonology, Heidelberg University Hospital, Heidelberg, Germany. [10]German Center for Infection Research (DZIF) and German Center for Cardiovascular Research (DZHK), Heidelberg, Germany. [11]JRG Developmental Origins of Pediatric Cancer, Hopp Children's Cancer Center Heidelberg (KiTZ) and German Cancer Research Center (DKFZ), Heidelberg, Germany

Pediatric high-grade glioma is a heterogeneous group of highly malignant tumors of the central nervous system, with a median overall survival of less than two years after diagnosis, demanding novel treatment options. One

innovative approach is gene therapy, which has so far been hampered for cancer treatment owing to the lack of a system targeting tumor cells specifically. To overcome this limitation, we established a novel strategy for gene therapy, combining tumor cell-specific adeno-associated virus (AAV) variants with oncogene-specific CRISPR-Cas nucleases. We screened 177 different Cas9/gRNA combinations targeting the genes encoding $H3^{K27M}$ or $BRAF^{V600E}$, and identified highly specific nucleases that edited the oncogenic allele but left the respective WT loci intact, which we validated by PCR amplicon sequencing. Next, we intravenously injected an AAV library engineered to encode its own capsid DNA into mice harboring patient-derived xenograft tumors driven by $H3^{K27M}$ or $BRAF^{V600E}$. After 21 days, we resected neoplasms and separated mCherry-labeled tumor cells from normal surrounding cells by fluorescence-activated cell sorting. Using the DNA from tumor cells as template, we generated a second AAV library, which was utilized in another round of *in vivo* selection. At the end of each screen, DNA from tumor cells, surrounding cells, and control tissues (liver and spleen) was analyzed by amplicon sequencing. Strikingly, we identified multiple AAV variants that were highly and recurrently enriched in the analyzed tumor tissues. We are currently validating these variants by intravenously injecting selected, GFP-encoding AAVs to tumor-bearing mice and by subsequently analyzing their distribution throughout the aforementioned tissues. We will combine oncogene-specific nucleases with these validated AAV variants and analyze their anti-tumoral efficacy in a preclinical setting. Furthermore, we plan to adapt this approach to allografted mice, evaluating its feasibility and efficacy in syngeneic models.

THER-02. PEDIATRIC BRAIN TUMOR CULTURES REVEAL DIFFERENTIAL SUSCEPTIBILITY TO FOUR ONCOLYTIC VIRUSES

Konstantinos Vazaios[1,2], Eftychia Stavrakaki[3], Lisette Vogelezang[3], Bernadette van den Hoogen[4], Rob C. Hoeben[5], Antonio E. Chiocca[6], William F. Goins[7], Esther Hulleman[1], Trudy Straetemans[2,8], Friso G. J. Calkoen[1], Jasper van der Lugt[1], Martine L. M. Lamfers[3]; [1]Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands. [2]Center for Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands. [3]Department of Neurosurgery, Brain Tumor Center, Erasmus Medical Center, Rotterdam, Netherlands. [4]Department of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands. [5]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands. [6]Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. [7]Department of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA. [8]Department of Hematology, University Medical Center Utrecht, Utrecht, Netherlands

INTRODUCTION: New therapeutic modalities such as Oncolytic viruses (OVs) are considered possible treatment options for pediatric brain tumors (PBTs) either as monotherapy or as adjuvants to immunotherapies. OVs specifically lyse tumor cells and can induce anti-tumor immune responses. Here, we evaluate the oncolytic potency of different clinically relevant OVs against various PBT entities. METHODS: The effect of four different OVs, Reovirus (R124), Newcastle Disease virus (NDV), Adenovirus (DNX-2401) and Herpes simplex virus-1 (rQNestin 34.5v.1), was assessed on patient-derived cell cultures belonging to four different PBT entities. Cell viability 5 days after virus treatment of diffuse midline gliomas (DMG n=6), atypical teratoid rhabdoid tumors (n=4), glioblastomas (n=1) and ependymomas (n=2) was measured using Cell Titer Glo assay to demonstrate the cytotoxic potency of each virus. RESULTS: Our screenings revealed that DNX-2401, rQNestin and NDV could infect and kill the majority of cell cultures (12 out of 13, 11 out of 13 and 11 out of 13, respectively). rQNestin34.5v.1 required lower amounts of infectious particles per cell (MedianEC50: 0.65±2.7) compared to NDV (3.5±1.7) and DNX-2401 (7.5±14.5), with DMGs being more sensitive for rQNestin34.5v.1 than non-DMGs. R124 was effective in only 6 out of 13 cultures, with DMGs being more resistant with EC50 > 100 (5 out of 6) compared to non-DMG cell lines with EC50 < 8 (5 out of 7). CONCLUSION: All cell lines revealed differential susceptibility to the 4 different OVs with at least one effective OV per cell line. Further analysis of transcriptome and methylome data might uncover genes and pathways which correlate with specific OV susceptibility and provide biomarkers for response prediction. Further investigation is ongoing to interpret how differential susceptibility affects OV-induced anti-tumor immunity.

INVITED SPEAKERS

INSP-01. WHAT TO DO WHEN YOU CANNOT RANDOMIZE? LEVERAGING HISTORICAL DATA IN EFFICIENT STUDY DESIGNS FOR PEDIATRIC NEURO-ONCOLOGY.

Arzu Onar-Thomas; St Jude Children's Research Hospital, Memphis, TN, USA

BACKGROUND: Randomized phase III studies represent the gold standard in clinical trial design for many good reasons. They control bias and the effects of known and unknown covariates on outcomes of interest. Recent examples from Children's Oncology Group Medulloblastoma studies have demonstrated their utility in providing insights that likely would not have been possible otherwise. However, experience with these trials also reaffirmed that large, randomized studies often take too long to keep up with the ever-changing landscape in pediatric Neuro-Oncology, and the rarity of these tumors is a significant barrier in utilizing such designs effectively. METHODS: Recent global efforts in pediatric Neuro-Oncology have led to rich, well annotated repositories that contain patient-level data. While these data suffer from the well-known limitations when used as sole comparison cohorts for ongoing studies, they also offer an opportunity to design more efficient studies in ultra-rare patient populations that trialists in pediatric neuro-oncology often face. Therefore, there is renewed effort in the statistical community in devising methodologies that can effectively utilize external data in the design of prospective studies. These approaches include incorporating external data as a supplement to a small fraction of patients randomized to standard of care arms and prospectively assessing similarity with an intent to minimize overall sample size. Others focus on patient selection methodologies from external controls with an intent to optimize matching between the retrospective and prospective cohorts to control for known covariates. Additional considerations include incorporating arms into the study that retain standard of care treatments to capture the magnitude of drift in outcome over time due to improved supportive care. CONCLUSIONS: While there are important limitations to designs based on external controls, judicious choice of design parameters and careful selection of controls could provide a viable alternative when rarity of patient populations make randomized designs infeasible.

INSP-02. WHO 2021 CLASSIFICATION OF CNS TUMORS

Pieter Wesseling; Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands

In line with recommendations of the cIMPACT-NOW consortium, the fifth edition of the WHO Classification of Tumors of the Central Nervous System (WHO CNS5 classification) is substantially different from the previous (revised 4th) edition. Salient changes include the separation of pediatric-type low- and high-grade diffuse gliomas from adult-type diffuse gliomas, refinement of the classification of ependymal tumors, and the addition of a newly recognized embryonal CNS tumors. Furthermore, for some tumors the name was changed. For example, diffuse midline glioma (DMG), H3K27M-mutant is now DMG, H3K27-altered (because there are H3-wildtype DMGs that do show loss of nuclear H3K27me3 staining and with a similar prognosis as DMGs, H3K27M-mutant), and supratentorial ependymoma, RELA fusion-positive was changed into ZFTA fusion-positive (as ZFTA ('zinc finger translocation associated', the new name for c11orf95) is the more frequent fusion partner in these tumors). The WHO CNS5 tumor classification certainly is an improvement, but it brings several (new) challenges as well. For example, for more CNS tumors it is now impossible to reach a state-of-the-art 'histomolecular' diagnosis in case molecular tools for assessment of essential diagnostic characteristics are not available. In those situations, adding NOS (not otherwise specified) to the histology-based diagnosis is the way to go. Furthermore, designing the optimal therapeutic management for newly defined tumor types is challenging. And while a more precise classification facilitates enrollment of more homogeneous populations of patients in clinical studies, the higher granularity of CNS tumor taxonomy makes it more difficult to perform studies on a large number of patients for particular tumor types. Still, one would like to think that patients suffering from a CNS tumor are better served by a more precise diagnosis because this allows for a better estimation of prognosis and, hopefully sooner than later, for a more tailored and effective therapeutic approach.

INSP-03. NEURONAL REGULATION OF GLIOMA PROGRESSION

Michelle Monje; Stanford University, Stanford, CA, USA

The nervous system regulates stem and precursor cell behavior across a range of tissues. In the central nervous system, neuronal activity is a critical regulator of development and plasticity. Activity-dependent proliferation of healthy glial progenitors, oligodendrocyte precursor cells (OPCs), and the consequent generation of new oligodendrocytes contributes to adaptive myelination. This plasticity of myelin tunes neural circuit function and contributes to healthy cognition. The robust mitogenic effect of neuronal activity on normal oligodendroglial precursor cells, a putative cellular origin for many forms of glioma, suggests that dysregulated or "hijacked" mechanisms of myelin plasticity might similarly promote malignant cell proliferation in this devastating group of brain cancers. Indeed, neuronal activity regulates initiation and promotes progression of gliomas in preclinical models. Crucial mechanisms mediating activity-regulated glioma progression include secretion of BDNF and the synaptic protein neuroligin-3 (NLGN3). NLGN3 induces multiple oncogenic signaling pathways in the cancer cell, and also promotes glutamatergic synapse formation between neurons and glioma cells. This synaptic and electrical integration of glioma into neural circuits