findings in the mouse brain, EZH2 is not expressed at any time point or spatial location within the developing human brain. Taken together, these data reject the above hypothesis, that EZH2 or H3K27M co-opt an endogenous PR2B inhibitory developmental program. Similarly, these results show that EZH2 is not expressed within the cell of origin for PFA or DMG. Further studies will seek to understand the endogenous function of EZH2 by further defining its normal expression pattern and function.

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

Jorge Lima1,2, Jorge Pinheiro1, Susana Nunes1, Ana Paula Fernandes1, Paula Soares1, José Carlos Machado2,3, Josue Pereira1, Maria João Gil da Costa1,3,4, Joana Lourenço4, Ipatimup, 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 pathology nomograms on pediatric neuro-oncology. 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, that were in HGG, and the latter in LGG. MYB and KIF1A rearrangements were also found in low grade gli/glioneuronal tumours, while HGG showed a more complex profile, with many cases harboring multiple alterations in EGFR, PDGFRA, ATRX, H3F3A, HIST1H2B, TP53, among others. A 16-year old patient with LGG (homogenous 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 medulloblastomas, the epigenetic profile had 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.

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

Adam Kravy1, Run Jin1, Chao Zhao1, Aruna Familiar1, Kathryn Weller1, Alexander Gromiha2, Ali Nasabzadeh3,4, Genevieve D’Angelo-Delcourt1. 1Children’s Hospital of Philadelphia, Philadelphia, PA, USA. 2Department of Biomedical and Health Informatics, Children’s Hospital of Philadelphia, Philadelphia, PA, USA. 3Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA, USA. 4Department 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 adaption via SLC1A5 inhibition results in growth-inhibitory and tumoricidal effects. Given their relatively higher expression in cancer versus normal tumor nutritional demands relative to non-cancerous cells, often through targeted radiation and application of available prognostic amino-acid PET imaging. However, the role of SLC transporters in pediatric brain cancer 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 (PBTAT). Using the expression of amino acid transporter genes across several in silico and in vivo models (Reactome: R-HSA-352230) we found that elevated expression of glutaminase transporters (SLC1A5, SLC7A5, SLC7A11, SLC38A5, SLC38A3) predicted shorter progression-free survival (PFS) in low-grade gliomas (LGGs) and poorer overall survival in pediatric epipdemoids, high-grade gliomas (HGGs), and medulloblastomas. Moreover, SLC1A5 was given the availability of imaging probes (18F-Fluoroglutamine and 18F-Fluclocline) for the corresponding amino acid transporter (ASCT2). Through transcriptome-based consensus clustering, we found that supratentorial, RELA fusion-positive ependymomas and the VEGFR transporter (ASCT2). Through transcriptome-based consensus clustering, we found that supratentorial, RELA fusion-positive ependymomas and 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 epipdemoid and medulloblastoma (p = 9.17e-9 and p = 0.032) in LGG. This analysis showed higher expression and network re-wiring 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.

VIRALGENE THERAPY AND OTHER NOVEL THERAPIES

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

Laura von Soosten1,2, Janina Haar1, Veronika Frehmann1, Stefan Heidelbrechter1, Julius Unенse zu Belzen1, Michael Jedruch1,2, Konstantin Okonechnikov3,4, Stefan M. Pfister1,2, Dirk Grimm1,2,10, Barbara Leuchs4, David Jones1, Lena M. Kutscher1, Marc Zuckermann1,2,10, Division of Pediatric Glioma Research, Hopp Children’s Cancer Center Heidelberg (KiTZ) and German Cancer Research Center (DKFZ), Heidelberg, Germany. 1Faculty of Biosciences, Heidelberg University, Heidelberg, Germany. 2Department of Infectious Diseases/Virology, Medical Faculty, University of Heidelberg, BioQuant, Heidelberg, Germany. 3Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany. 4Synthetic Biology Group, BioQuant Center, University of Heidelberg, Heidelberg, Germany. 5Digital Health Center, Berlin Institute of Health (BIH) and Charité University Medicine, Berlin, Germany. 6European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; Division of Pediatric Neurooncology, Hopp Children’s Cancer Center Heidelberg (KiTZ) and German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany. 7Division of Pediatric Neurooncology, Hematology, Immunology and Pulmonology, Heidelberg University Hospital, Heidelberg, Germany. 8Department of Infectious Diseases/Virology (DZIF) and German Center for Cardiovascular Research (DZHK), Heidelberg, Germany. 9JR 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 adenovirus-associated virus (AAV) vari-
ants with oncogene-specific CRISPR-Cas nucleases. We screened 177 differ-
ent Cas9/gRNA combinations targeting the genes encoding H3K27m or BRAFV600E, and identified highly specific nucleases that edited the oncogenic area but left the respective wild type intact, which we validated by PCR or amplicon sequencing. Next, we intravenously injected an AAV library engi-
erized to encode its own capsid DNA into mice harboring patient-derived xenograft tumors driven by H3K27m or BRAFV600E. After 21 days, we re-
sected neoplasms and separated mCherry-labeled tumor cells from surrounding cells by fluorescence-activated cell sorting. Using the DNA from tumor cells as template, we generated a second AAV library, which was util-
ized 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 tis-
ses. 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 Vazanos1,2, Etychka Stavrakas1,2, Lisaette Vogelezang1, Bernadette van den Hoogen1, Rob C. Hoehen1, Antonio E. Chioaca1, William F. Goins1, Esther Hulleman2, Trudy Straetemans1,3, Frédéric J. Calle1, Jasper van der Lugt1, Liesl M. Lamers1,2,
1Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands.
2Center for Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands.
3Department of Neurosurgery, Brain Tumor Center, Erasmus Medical Center, Rotterdam, Netherlands.
Department of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands.
4Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands.
5Department of Neurosurgery, Brigham and Women’s Hospital/Harvard Medical School, Boston, Massachusetts, USA.
6Department of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.
7Department 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 (rQ1Nestin3 34.5v.1), we tested in patient-derived cell cultures belonging to four different PBT entities. Cell viability 5 days after virus treatment of diffuse midline gliomas (DMG), atypical teratoid rhabdoid tumors (n=4), glioblastomas (n=1) and ependymomas (n=2) was measured using Cell Titer Glo assay to demonstrate the cytopathic effect of each virus. RESULTS: Our screenings revealed that DNX-2401, rQ1Nestin NDV 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). rQ1Nestin 34.5v.1 required lower amounts of infectious particles per cell (Median±SE: 0.6±0.72) compared to NDV (3.5±1.7) and DNX-2401 (7.3±1.45), with DMGs being more sensitive for rQ1Nestin34.5v.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 tolerated the viruses, with DMGs being more resistant with EC50 > 100 (5 out of 6) compared to non-DMGs being more sensitive to NDV (3.5±1.7) and DNX-2401 (7.5±14.5), with DMGs being more sensitive to NDV (3.5±1.7) and DNX-2401 (7.5±14.5), with DMGs being more sensitive to NDV (3.5±1.7) and DNX-2401 (7.5±14.5), with DMGs being more sensitive to non-DMGs. R124 was effective in only 6 out of 13 cultured cell lines. Further analysis of transcriptome and methylome data might revealed differential susceptibility to the 4 different OVs with at least one effective OV to non-DMG cell lines with EC50 < 8 (5 out of 7). CONCLUSION: All cell lines tolerated the viruses, with DMGs being more resistant with EC50 > 100 (5 out of 6) compared to non-DMGs being more sensitive to NDV (3.5±1.7) and DNX-2401 (7.5±14.5), with DMGs being more sensitive to non-DMGs. R124 was effective in only 6 out of 13 cultured cell lines. Further analysis of transcriptome and methylome data might revealed differential susceptibility to the 4 different OVs with at least one effective OV to non-DMG cell lines with EC50 < 8 (5 out of 7).

INVITED SPEAKERS

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

Aaron Onlar-Thomas1,2 St Jude Children’s Research Hospital, Memphis, TN, USA

BACKGROUND: Randomized phase III studies represent the gold standard in clinical research 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, in the face 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 for certain research efforts in pediatric oncology. OVs 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 tumors in pediatric neuro-oncology often face. Therefore, there is renewed effort in the statistical community 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 prospective assessing similarity with an intent to minimize overall sample size. Others focus on patient selection methodologies from external controls with an intent to op-
timize 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 popula-
tions make randomized designs infeasible.

INSPE-02. WHO 2021 CLASSIFICATION OF CNS TUMORS

Pieter Wesseling1, 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 named, the name was changed. For example, a different mutated 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, H3K27-mutant), and supratentorial ependymoma, RELA fusion-positive was changed into ZFTA fusion-positive (as ZFTA (zinc finger translation associated), the new name for c1tort53 is the more frequent fusion partner in these tumors). The WHO CNS5 tumor classification certainly is an improvement, but it brings several (new) chal-
enges as well. For example, for more CNS tumors it is now impossible to reach a clear-cut-of-the-art ‘histo-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 very challenging. And while a more precise classification facilitates enrollment of more homogeneous popula-
tions 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 rare tumor types. Still, one would like to think that pa-
tients suffering from a CNS tumor are better served by a more precise diag-

osis because this allows for a better estimation of prognosis and, hopefully sooner than later, for a more tailored and effective therapeutic approach.

INSPE-03. NEUROURAL REGULATION OF GLIOMA PROGRESSION

Michelle Monje1, 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 brain progenitor cells, oligodendrocyte precursors and non-neural precursors is the more frequent fusion partner in these tumors). The WHO CNS5 tumor classification certainly is an improvement, but it brings several (new) chal-
enges as well. For example, for more CNS tumors it is now impossible to reach a clear-cut-of-the-art ‘histo-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 very challenging. And while a more precise classification facilitates enrollment of more homogeneous popula-
tions 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 rare tumor types. Still, one would like to think that pa-
tients suffering from a CNS tumor are better served by a more precise diag-
nosis because this allows for a better estimation of prognosis and, hopefully sooner than later, for a more tailored and effective therapeutic approach.

NEURO-ONCOLOGY • JUNE 2022