

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 H3K27M or BRAF<sup>V600E</sup>, 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 H3K27M or BRAF<sup>V600E</sup>. 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

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**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 (rQNestin34.5v.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.

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**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

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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

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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