IMMU-14. COMPUTATIONAL DECONVOLUTION OF TUMOR-INFILTRATING IMMUNE COMPONENTS IN PEDIATRIC NERVOUS SYSTEM TUMORS

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Introduction: In the last decade, checkpoint inhibitor-based immunotherapy has been a groundbreaking development in the treatment of cancer. However, only a subset of patients treated with immune checkpoint inhibitors show long-lasting clinical benefit. Studies showed the tumor immune microenvironment (TME) as a particularly important factor influencing treatment response, critical for the design of other or combinatorial immunotherapy treatment strategies. Extensive research has been performed in the adult cancer field to unravel its immunogenomic aspects. However, in pediatric cancer this insight into tumor-infiltrating immune components is still lacking. This study aims to provide insight into the landscape of the immune microenvironment in pediatric primary nervous system tumors. Methods: Bulk RNA-seq data of 936 pediatric primary solid tumors acquired from multiple international initiatives including Therapeutically Applicable Research To Generate Effective Treatments (TARGET), the International Cancer Genome Consortium (ICGC) and the Children's Brain Tumor Tissue Consortium (CBTTC) were included in this study. We applied computational tumor immune microenvironment deconvolution, repurposed RNA-seq data to recover infiltrating T- and B-cell clonotypes and studied checkpoint gene expression across pediatric neural tumors. Results: Among pediatric neural tumors, embryonal tumors with multilayered rosettes (ETMR) and medulloblastomas (MB) were least immune infiltrated. Neuroblastomas (NBL) had the highest T-cell infiltration among pediatric cancers, while atypical teratoid/rhabdoid tumors (ATRT) had the highest levels of CD8 T cell infiltration among pediatric CNS tumors. While tumor mutational burden (TMB) was associated with immune cell infiltration in adult lung cancers and melanomas, we found no significant associations in pediatric cancers. The majority of NBL samples expressed *LAG3*, but ~10% of samples had elevated levels of *TIM3* gene expression, suggesting a distinct mode of immunosuppression in this subset.

IMMU-15. QUANTIFYING INTRATHECAL DRUG DELIVERY UTILIZING PROGRAMMABLE VENTRICULOPERITONEAL SHUNTS

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Background: Programmable ventriculoperitoneal (pVP) shunts are increasingly utilized for intraventricular chemotherapy, radioimmunotherapy, and/or cellular therapy. Shunt adjustments allow optimization of thecal space drug concentrations with minimization in the peritoneum. Drug delivery quantification using several types of pVP shunts has not been reported. Methods: We performed a retrospective analysis on patients with CNS tumors and pVP shunts at Memorial Sloan Kettering Cancer Center from 2003–2020, noting shunt model. CSF flow through the pVP shunt was evaluated using In-111-DTPA scintigraphy at approximately 4 hours and 24 hours after injection. pVP shunts were calibrated pre-injection to minimize peritoneal flow and re-calibrated to baseline setting 4–5 hours following injection. Scintigraphy studies quantified ventricular-thecal and peritoneal drug activity at these 2 time points. Results: Twenty-one CSF flow studies were administered to 15 patients, ages 1–27 years. Diagnoses included medulloblastoma (N=10), metastatic neuroblastoma (N=3), pineoblastoma (N=1), and choroid plexus carcinoma (N=1). pVP shunt models included Aesculap Miethke proGAV (N=3), Aesculap Miethke proGAV2.0 (N=3), Codman HAKIM (N=2), Codman Certas Plus (N=1), Medtronic STRATA (N= 5), and Sophysa Polaris (N= 1). All 21 studies (100%) demonstrated ventriculothecal drug activity. 29% (6 of 21) of the studies had no peritoneal uptake visible by imaging. 73% (16 of 21) of the studies had minimal peritoneal uptake (<12%), and 24% (5 of 21) demonstrated moderate peritoneal uptake (12–37%). pVP shunt models measuring minimal to no peritoneal uptake included: Aesculap Miethke proGAV (N=2), Aesculap Miethke proGAV2.0 (N=3), Codman HAKIM (N=2), Codman Certas Plus (N=1), Medtronic STRATA (N= 3), and Sophysa Polaris (N= 1). Conclusions: Successful drug delivery to the ventriculo-thecal space can be accomplished using pVP shunts: 80% of studies have minimal (<12%) peritoneal drug activity. Though efficacy varies by shunt model, low numbers preclude conclusions regarding model superiority. CSF flow scintigraphy studies reliably assess drug distribution.

IMMU-16. TARGETING GLYPICAN 2 (GPC2) ON PEDIATRIC MALIGNANT BRAIN TUMORS WITH MRNA CAR T CELLS

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Glypican 2 (GPC2) is a cell-surface oncoprotein initially identified in neuroblastoma, retinoblastoma, and medulloblastoma as an ideal target for immunotherapy (Cancer Cell, 2017). Here we evaluated *GPC2* expression across the spectrum of pediatric brain tumors using RNA sequencing from specimens in the Children's Brain Tumor Network (CBTN). High *GPC2* expression, defined as >10 FPKM, was found in 100% of embryonal tumors with multilayered rosettes (ETMRs) (n=6), 95% of medulloblastomas (n=122), 86% of other embryonal tumors (n=21), 50% of choroid plexus carcinomas (n=4), 42% of high grade gliomas (HGG) (n=117), and 37% of diffuse midline gliomas (DMG) (n=65). Within medulloblastoma subtypes, group 4 tumors had the highest expression, and within the HGG tumor cohort H3.3 G34 mutated gliomas had the highest *GPC2* expression. High GPC2 protein expression was validated with medulloblastoma and HGG/DMG primary tumors and cell lines using IHC, Western blot, and flow cytometry. We next developed two potent CAR T cell constructs using the D3 specific scFv directed against GPC2 for testing in brain tumor models. GPC2-directed CAR T cells were tested *in vitro* against medulloblastoma and HGG cells lines, and *in vivo* using two patient-derived medulloblastoma xenograft models: Rcmb28 (group 3) and 7316-4509 (group 4). GPC2-directed mRNA CAR T cells induced significant GPC2-specific cell death in medulloblastoma and HGG cellular models with concomitant T cell degranulation compared to CD19-directed mRNA CAR T cells. *In vivo,* GPC2-directed mRNA CAR T cells delivered locoregionally induced significant tumor regression measured by bioluminescence after 4–6 intratumoral infusions of 4 x 10^6 CAR T cells ($p<0.0001$ for Rcmb28, $p<0.05$ for 7316-4509). No GPC2-directed CAR T cell related toxicity was observed. GPC2 is a highly differentially expressed cell surface protein on multiple malignant pediatric brain tumors that can be targeted safely with local delivery of mRNA CAR T cells.

IMMU-17. USE OF MRNA FOR SAFE AND EFFECTIVE GD2-DIRECTED CAR T CELLS TO TREAT DIFFUSE MIDLINE GLIOMAS

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Chimeric antigen receptor (CAR) T cells targeting the disialoganglioside GD2 have garnered interest as an effective therapeutic for treating diffuse midline glioma (DMG). However, prior studies raised significant concerns of neurotoxicity and fatality when using virally transduced CAR T cells against these midline tumors. Building upon our prior work optimizing mRNA for use in CAR T cells (Hum Gen Ther, 2019), we hypothesized transient GD2-directed mRNA CAR T cells could be successfully employed for safe and effective treatment of DMG. GD2-directed CAR T cells were created using mRNA encoding the 14G2a single chain variable fragment paired with 41BB and CD3-zeta co-stimulatory domains and transfected into human T cells. GD2-directed CAR T cells were tested against a panel of DMG cell lines and two murine xenograft models of DMG: 7316-6349 and SU-DIPG13P*. In all DMG cellular models, GD2-directed mRNA CAR T cells induced significant tumor cell death compared to CD19-directed mRNA CAR T cell controls. *In vivo,* mRNA CAR T cells were delivered locoregionally using an indwelling infusion catheter to allow for repeated dosing. Four intratumoral doses of 5 x 10^6 GD2-directed mRNA CAR T cells induced significant tumor regression measured by bioluminescence in DMG model 7316-6349 ($p<0.0001$). In addition, GD2-directed mRNA CAR T cells prolonged survival of mice harboring the aggressive DMG model SU-DIPG13P* by 61% (mean survival 29 days versus 18 days, $p<0.01$) following four intratumoral doses of 4 x 10^6 CAR T cells. No GD2-directed CAR T cell treatment-related deaths or toxicities were observed. These data highlight the utility of