were monitored for toxicity and response. RESULTS: Autologous TAAT products were successfully manufactured from 28 patients. Using IFN-γ ELISPOT assay, 10/11 evaluable products had specificity for 1-3 TAAs. 25 patients received TAA-T (6 in Stratum A and 19 in Stratum B) and completed the 45-day safety monitoring period. Twenty-four (96%) had no dose limiting toxicities (DLT), but 1 (4%) patient with DIPG experienced a DLT related to potential immune-mediated pseudoprogression. Median overall survival for patients with DIPG (Stratum A) was 14 months (range, 6-32 months). Median progression-free survival (PFS) for Stratum B patients was 8 months (range, 2-26+ months), which exceeded their preceding median duration of disease stability of 2 months (range, 1-5 months). Plasma cytokine profiles demonstrated infusion-related immune cytokine responses. CONCLUSIONS: In summary, TAAT had a favorable toxicity profile (4%) especially compared to CAR-T therapy and may elicit anti-tumor immune responses that contribute to prolonged survival. Immunobiology studies and response assessments are ongoing for both strata. Based on these encouraging preliminary results, we have added a stratum that includes prescribed lymphodepletion pre TAA-T administration at a cell dose of 8x107/m2. Further, we plan to add an additional stratum to allow direct administration of TAA-T into the CNS via an Ommaya reservoir.

IMMU-20. EFFECTIVE CAR-T CELL MEDULLOBLASTOMA THERAPY IN AN IMMUNOCOMPETENT MOUSE MODEL

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Immunotherapy with chimeric antibody receptor (CAR) T cells is effective for previously incurable hematologic cancers and may transform treatment for refractory brain tumors. However, CAR-T cell therapy for solid tumors has not yet been as successful as for leukemias, and animal models are needed to improve implementation. At present, preclinical studies of CAR-T cell therapy for brain tumors have typically used exogenous tumors, xenografted into immunocompromised mice, because primary mouse brain tumors do not express antigens that match brain tumor-specific antigens in humans. To advance preclinical development of CAR-T brain tumor therapy, we engineered mice to develop medulloblastomas that express B7-H3, an antigen specifically expressed on human medulloblastomas and other pediatric brain tumors. We show that treating these tumors with B7-H3-directed CAR-T cells provokes anti-tumor responses both in vitro and in vivo. Administering B7-H3 CAR-T cells by intracranial injection increased the event-free survival time of mice with medulloblastoma, in a dose-dependent manner. CAR-T cell treatment was not curative as an isolated intervention, suggesting that cure will require pairing with surgical resection and additional adjuvant therapy. Our model presents new opportunities to study the mechanisms of CAR-T cell efficacy and recurrence in an immunocompetent host with intact vasculature and blood-brain barrier. Our ongoing studies using scRNA-seq will allow us to define therapy-induced changes in tumor cells, CAR-T cells and cells of the tumor microenvironment and to test new T-cell modifications and combinations of therapeutic modalities, toward a goal of optimizing CAR-T cell therapy for pediatric brain tumors.

IMMU-21. TARGETING THE ADENOSINERGIC IMMUNE SUPPRESSION PATHWAY IN HIGH GRADE GLIOMA SYNERGIZES WITH INNATE IMMUNE CHECKPOINT BLOCKADE

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Immune response in the tumor microenvironment is modulated by the conversion of eATP to AMP to adenosine via the ecto-enzymes CD39 and CD73. Overexpression of CD73 in tumors leads to an increase in extracellular adenosine concentration and decrease in eATP, resulting in immune suppression. In this study, CD73 knockouts were created in adult and pediatric glioma cell lines (DIPG17, T3691, BT245) and used to quantify the effects of CD73 knockout on macrophage phagocytosis in the presence of extracellular AMP. In cell line DIPG17, CD73 knockout significantly increased phagocytosis with and without external AMP compared to the WT. In the WT condition, addition of AMP significantly reduced phagocytosis, while this decrease was not significant in the CD73-KO. The addition of anti-CD47 antibody significantly increased phagocytosis with external AMP in the WT condition. Similar results were obtained with cell line T3691. However, CD73 knockout combined with anti-CD47 treatment and external AMP showed significantly greater phagocytosis than the WT condition. This suggests that a combination of anti-CD73 and anti-CD47 treatment may be more effective in the tumor microenvironment. Lastly, in cell line BT245, CD73 knockout significantly increased phagocytosis as seen in the other cell lines. However, no significant difference was observed between WT and CD73-KO with extracellular AMP or anti-CD47. In vivo studies were conducted in orthotopic xenograft mouse models with DIPG17 CD73 knockout cells. Combination treatment with anti-CD47 antibody significantly decreased tumor burden and prolonged survival in the CD73-KO tumors compared to anti-CD47 treated DIPG13-WT tumors. We conclude that the CD73 adenosinergic pathway and the CD47-SIRPα pathway may present a target for immunotherapy in pediatric and adult gliomas.

IMMU-22. SAFELY TARGETING GD2 IN THALAMIC DIFFUSE MIDLINE GLIOMA WITH MRNA CAR T CELLS

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Chimeric antigen receptor (CAR) T cells targeting the disialoganglioside GD2 have shown promise as a therapeutic for diffuse midline glioma (DMG). However, prior studies raised significant concerns of neurotoxicity and fatality when using virally transduced CAR T cells against midline thalamic tumors. Building upon our prior work optimizing mRNA for use in CAR T cells (Hum Gen Ther, 2019), we hypothesized repeated dosing of transient GD2-directed mRNA CAR T cells could be employed for safe and effective treatment of thalamic 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. CAR T cells were tested against the murine thalamic DMG xenograft 7316-6349 via locoregional delivery with an indwelling infusion catheter for repeated dosing. The previously reported fatal neurotoxicity observed in mice using lentiviral CAR T cells could be recapitulated with aggressive dosing. Four doses of 5 x 10^6 mRNA CAR T cells delivered intratumorally twice a week resulted in median overall survival of 9 days for GD2-treated mice compared to >30 days for CD19-treated controls ($p<0.01$). This toxicity could be avoided by decreasing the dose and timing of infusions to 2 x 10^6 mRNA CAR T cells delivered once weekly. Bioluminescent imaging showed regression of tumor in GD2-treated mice compared to CD19-treated controls (radiance fold change -3 x10^6 versus +20x10^6 p/sec/cm2/sr, $p<0.01$). Notably, non-tumor bearing mice treated with GD2-directed CAR T cells quickly developed fatal neurotoxicity within 14 days, suggesting on-target/off-tumor effect of the CAR T cells and a very narrow therapeutic window in the brain. These data highlight the utility of titratable mRNA-based CAR T cell therapy for CNS tumors and establish GD2-directed mRNA CAR T cells as a safe and effective method for treating thalamic DMG.

IMMU-23. NOVEL GENE-EDITED CAR-T CELL THERAPY AGAINST DIFFUSE INTRINSIC PONTINE GLIOMA

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BACKGROUND: We identified high expression of CD99 in DIPG tumors and developed a CAR using our newly identified single chain variable fragment (scFv) targeting CD99 incorporating a 4-1BB co-stimulatory domain. This CD99 CAR demonstrated the ability to dramatically shrink the established orthotopic DIPG tumor, however tumor recurrence remains an obstacle to cure, due to a loss of the CAR-T cells as they also express the target antigen, CD99 (fratricide). To overcome this obstacle, we modified these CAR-T by editing out CD99. METHODS: CD99 was knocked-out from the human T cells using CRISPR-cas9 gene-editing and subsequently transduced with our CD99 CAR-encoding virus, and isolated the pure population of CD99KO T-cells. These novel, gene-edited T-cells expressing CD99 CAR ("CD99KO CARs") and the un-edited ones ("CD99 CAR") were tested for tumor-lysis function when co-cultured with DIPG cells. DIPG tumor-bearing mice infused with a one-time dose of CD99KO CAR-T cells or CD99 CAR- or CD19 control CAR-T cells and were monitored for changes in the tumor burden. At the endpoint spleen and bone marrow were isolated to test for CAR+ cell persistence. RESULTS: The CD99KO CAR-T cells demonstrated effective tumor-lysis when co-cultured with DIPG cells. CD99KO CAR-T cells targeting CD99 showed complete clearance of DIPG tumor in orthotopic DIPG mouse models, and no tumor recurrence was seen well-beyond the time frame of expected tumor recurrence after treatment with un-edited CD99 CAR-T cells. There was an un-precedented increase in the xenograft survival, > 200 days, in mice treated with CD99KO CARs and at which time point sustained persistence of CAR+ cells were evident in the animal spleen and bone marrow. CONCLUSIONS: We have generated a new and promising CAR-T cell therapy that is effective against DIPG with enhanced persistence in animal models which is critical for clinical translation.