expansion. We found that shortening ex vivo expansion of either TCR-specific murine Th17 cells or human CAR Th17 cells licenses the cell product to eradicate large tumors in low doses and generate long-lived memory against tumor. Therapeutic Th17 cells induce the systemic release of IL-6, IL-17, GM-CSF, and MCP-1 among other cytokines in tumor-bearing hosts, reminiscent of clinical cytokine release syndrome. As the toxicity of cytokine release is managed in patients through IL-6 blockade, we addressed the impact of IL-6 on efficacy and durability of Th17 cell therapy. We hypothesized that IL-6, induced by Th17 cells, was fueling the durable memory properties of this cell product.

Methods Th17 cells were expanded ex vivo using the TRP-1 transgenic mouse model in which CD4+ T cells express a TCR that recognizes tyrosinase-related protein 1 on melanoma. Naïve CD4+ T cells were polarized to the Th17 phenotype and infused into mice with B16F10 melanoma after a nonmyeloablative total body irradiation (5 Gy) preparative regimen.

Results IL-6 blockade, targeting either IL-6R or neutralization of the cytokine, did not significantly impact the primary immune response of adoptively transferred Th17 cells against tumor. However, administering IL-6 blockade acutely after Th17 transfer resulted in a higher incidence of tumor relapse upon secondary tumor challenge, thereby compromising long-lived antitumor immunity. Mounting a secondary response to tumor was dependent on CD4+ T cells, but not CD8+ T cells, persisting in the host. Mechanistically, IL-6 blockade reduced pSTAT3 and Bcl2 in transferred T cells but did not greatly impact the concentration of other systemic cytokines. As a small fraction of Tregs remain in the Th17 cell product ex vivo, we examined the engraftment of those Tregs after transfer. IL-6 was critical to suppress engraftment of FoxP3+ donor T cells from the CD4+ T cell product. Thus, IL-6 promoted robust tumor infiltration by donor effector over regulatory cells for early Th17 cells relative to cell products expanded longer durations ex vivo.

Conclusions Overall, short-term expanded Th17 cells uniquely induced IL-6 unlike Th17 cells expanded longer ex vivo. IL-6 promoted Th17 survival, reduced engraftment of tumor-specific Tregs, and was critical to durable memory. This work may suggest that the universal strategy to inhibit IL-6 during tumor cell lines. In breast cancer in vivo xenograft model, both unedited (MUC1C CAR-T) and edited (P-MUC1C-ALLO1) MUC1C CAR-T eliminated established, triple negative MDA.MB.468 triple negative breast cancer model and the OVCAR3 disseminated ovarian cancer model.

Results Specific degranulation of transiently-expressing CAR+ T cells was observed against multiple tumor cells, with no observable activity against normal human primary cells. In assay of stable P-MUC1C-101 CAR-T cells, more than 95% expressed CAR, and were comprised of an exceptionally high percentage of T stem cell memory (TSCM) cells. To produce allogeneic cell products, multiplex editing of both TRBC and B2M was performed with the CasCLOVER™ Site-Specific Gene Editing System to reduce or eliminate GvHD and host versus graft alloreactivity, respectively. To determine in vivo antitumor efficacy of MUC1C CART cells, we employed the MDA.MB.468 triple negative breast cancer model and the OVCAR3 disseminated ovarian cancer model.

Conclusions P-MUC1C-ALLO1 is Poseida’s allogeneic CAR TSCM product that has a potential to treat multiple MUC1-expressing indications. P-MUC1C-ALLO1 displayed in vitro specificity for tumor vs normal cells, and in vivo efficacy against xenograft models of breast and ovarian cancer. We anticipate an IND filing and initiation of a Phase 1 clinical trial in 2021.
metastatic gastric cancers. In the context of the efficacy of GUCY2C-directed chimeric antigen receptor (CAR)-T cells against metastatic colorectal cancer in animal models, we hypothesized that this adoptive cell therapy may be effective against metastatic gastric cancer.

Methods Here, we explored the efficacy of GUCY2C-directed CAR-T cells for gastric cancer in a patient derived xenograft (PDX) tumor model. Also, we interrogated translational GUCY2C biomarker assays using RT-qPCR, immunoblot analysis, and immunohistochemistry (IHC) for the intended purpose of identifying patients whose tumors express GUCY2C and could benefit from GUCY2C-directed CAR-T cell therapy.

Results GUCY2C-directed CAR-T cells significantly reduced subcutaneous T84 colorectal tumor growth, producing a 5-fold reduction in tumor volume, compared to control treated tumors. GUCY2C-directed CAR-T cells produced no response in tumors produced from the GUCY2C-deficient colorectal cancer cell line, SW480. Importantly, GUCY2C-directed CAR-T cells controlled gastric cancer PDX growth, maintaining a >12-fold reduction in tumor volume compared to control and in some cases produced complete tumor elimination. Furthermore, IHC based assays, indicate that antibodies developed in our laboratory may be suitable for development of a companion diagnostic for GUCY2C-directed CAR-T cells. Indeed, the commercial polyclonal antibody demonstrated robust, non-specific staining regardless of tissue type or GUCY2C mRNA profile, while novel monoclonal antibodies produced in our laboratory primarily detected protein localized to the membrane of glandular epithelial cells, demonstrating antigen specificity, and indicating their potential for further development in diagnostic companion assays to identify gastric cancer patients who may benefit from GUCY2C-directed CAR-T cell therapy.

Conclusions GUCY2C-directed CAR-T cells prevented the growth of, and at times eliminated, a subcutaneous gastric cancer PDX model. In the context of previously established safety in mouse models, additional studies defining the efficacy of GUCY2C-directed CAR-T cells in gastric cancer models may allow future translation of this therapy to patients with advanced gastric cancers. Concurrent development of a novel companion diagnostic IHC assay would permit identification of the ~50% of gastric cancer patients whose tumors express GUCY2C and could benefit from this therapy.

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Ethics Approval This study was approved by the Thomas Jefferson University Institutional Review Board (# 14.0204) and the Institutional Animal Care and Use Committee (Protocol # 01529).

REFERENCES

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123 NATURAL KILLER CELLS ENGINEERED WITH AN INDUCIBLE, RESPONSIVE GENETIC CONSTRUCT TARGETING TIGIT AND CD73 TO RELIEVE IMMUNOSUPPRESSION WITHIN THE GBM MICROENVIRONMENT

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Background Solid tumors such as GBM are particularly difficult to treat, being largely resistant to traditional treatments, fueling interest in alternative treatment approaches, including cell-based immunotherapy. Natural killer (NK) cells have emerged as promising effectors to target GBM through genetic modifications and ex vivo manipulation. However, immunosuppressive conditions within the tumor microenvironment (TME) further complicate NK cell-based treatments. Specifically, within the TME tumor cells release of high levels of ATP extracellularly. While intracellular ATP is necessary for cell metabolism, extracellular ATP is converted into adenosine (ADO) by ectonucleotidases CD39 and CD73, both overexpressed on GBM. Extracellular ADO induces immunometa- bolic suppression of NK cells through binding with A2A adenosine receptors (A2ARs) on NK cells, suppressing cytokine secretion, proliferation, and other functional activities. Adding to the suppression of NK cells is the interaction between CD155, expressed highly on GBM and other solid tumors, and T cell immunoreceptor with Ig and ITIM domains (TIGIT) expressed on NK cells. This interaction signals inhibition of NK cell cytolytic function, allowing for cancer cell immune-evasion.

Methods To restore impaired NK cell anti-tumor activity, we have engineered NK cells to concomitantly target CD155 and CD73-induced immunosuppression on GBM using a tumor-responsive genetic construct. The construct is capable of blocking the immunosuppressive CD155/TIGIT interaction,