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

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 immunosuppression 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,
and, upon binding, release a CD73-blocking scFv to inhibit the accumulation of extracellular ADO and mitigate immunosuppression of NK cells. Such localized response enhances specificity and reduces off-target effects of NK-based targeting. Results Primary NK cells were successfully electroporated to express our synthetic TIGIT-synNotch construct, as evidenced by increased expression levels of TIGIT (% and MFI) (figure 1). To evaluate the functionality of engineered NK cells against GBM targets, we tested the cytotoxicity of our engineered NK cells against a primary, patient-derived GBM cell line, GBM43. Overall, cytolytic function of engineered NK cells against GBM was significantly higher than that of non-engineered NK cells, with or without CD73 (10 ug/mL) and TIGIT (50 ug/mL) antibodies, for E:T ratios of 5:1 and 10:1 (figure 2), demonstrating the functional efficacy of our genetic construct. Further, engineered NK cells (T-PNK) expressed significantly higher levels of CD107a in response to GBM43 stimulation than non-engineered PNK at E:T ratios 2.5:1 and 10:1 (figure 3).

Conclusions Overall, we have shown that co-targeting CD155 and CD73 in a localized, responsive manner can dampen immunosuppression and significantly enhance the killing potential of engineered NK cells against aggressive patient-derived GBM tumors.

REFERENCES

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124 OPTIMIZING THE GENERATION FROM UMBILICAL CORD BLOOD OF ‘OFF-THE-SHELF’ CD19-CHIMERIC ANTIGEN RECEPTOR (CAR) EXPRESSING T CELLS
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Background T lymphocytes expressing CD19-chimeric antigen receptor (CAR) showed the improvement of overall survival of patients with B-cell malignancies. Allogeneic CAR-T cells can overcome the limitation of the availability of patient’s lymphocytes, reducing the waiting time for the treatment and decreasing the cost of manufacturing. This study is aimed at the optimizing the generation of ‘off-the-shelf’ CAR-T cells utilizing Umbilical Cord Blood (UCB) to isolate T lymphocytes.

Methods UCBs have been collected at the time of childbirth from volunteer pregnant women at Sidra Medicine. Following the magnetic depletion of non-T cells, UCB-T lymphocytes were activated in vitro for 48 hr. by agonistic CD3/CD28 mAbs either conjugated to magnetic beads (Dynabeads) or to a colloidal polymeric nanomatrix (TranAct; Miltenyi Biotec). T cells generated in vitro were either i. untransduced (UT), or transduced with lentiviral encoding for ii. CD19-CD28z or iii. CD19-4-1BBz CARs. N=32 T cell cultures have been generated from fresh UCB (N=3) and, as control, from the peripheral blood lymphocytes of healthy donors (PBL; N=3) and used for deep phenotype analyses (28 markers) at different time points (Day +9 and Day+14) of the in vitro culture. Cytokines, perforin and granzyme B release (EliSpot or