CAR-M are professional antigen presenting cells, we developed an immunocompetent animal model to evaluate the potential for induction of a systemic anti-tumor immune response.

Methods Murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adeno viral vector Ad5F35. CAR-M were phenotypically and functionally evaluated in vitro and in syngeneic models. To evaluate CAR-M efficacy in an immunocompetent animal model, BALB/c mice were engrafted with CT26-HER2+ tumors (single-tumor model) and were treated with intratumoral CAR-HER2 or untransduced (UTD) macrophages. To evaluate epitope spreading, we simultaneously engrafted BALB/c mice with CT26-HER2+ and CT26-Wt tumors on opposite flanks (dual-tumor model), and treated mice with CAR-M or controls into the CT26-HER2+ tumor only. Peripheral and tumor-infiltrating immune cells were phenotypically and functionally characterized.

Results In addition to efficient gene delivery, Ad5F35 transduction promoted a pro-inflammatory (M1) phenotype in murine macrophages. CAR-M, but not control UTD macrophages, phagocytosed HER2+ target cancer cells. Anti-HER2 CAR-M eradicated HER2+ murine CT26 colorectal and human AU565 breast cancer cells in a dose-dependent manner. CAR-M increased MHC-I and MHC-II expression on tumor cells and promoted tumor-associated antigen presentation and T cell activation. In vivo, CAR-M treatment led to tumor regression and improved overall survival in the CT26-HER2+ single-tumor model. In the dual-tumor model, CAR-M treatment cleared 75% of CT26-HER2+ tumors and inhibited the growth rate of contralateral CT26-WT tumors, demonstrating an abscopal effect. CAR-M treatment led to increased infiltration of intratumoral CD4+ and CD8+ T, NK, and dendritic cells – as well as an increase in T cell responsiveness to the CT26 MHC-I antigen gp70, indicating enhanced epitope spreading. Given the impact CAR-M had on endogenous T cell immunity, we evaluated the combination of CAR-M and anti-PD1 in the CT26-HER2 model and found that the combination further enhanced tumor control and overall survival.

Conclusions These results demonstrate that CAR-M therapy induces epitope spreading via activation of endogenous T cells, orchestrating a systemic immune response against solid tumors. Moreover, our findings provide rationale for the combination of CAR-M with immune checkpoint inhibitors. The anti-HER2 CAR-M CT-0508 will be evaluated in an upcoming Phase I clinical trial.

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133 DEVELOPMENT OF NOVEL CELLULAR THERAPEUTICS FOR METASTATIC AND PRIMARY CNS MALIGNANCIES
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Background Treatment of solid tumors with cell therapeutics will require optimal T cell persistence, fitness, and trafficking. Heterogeneous solid tumors will also have to be attacked through multiple antigens simultaneously in order to prevent resistance linked to loss of antigen expression. Here we use chimeric antigen receptor (CAR) T cells that secrete bridging proteins that act as CAR-T engagers to create an optimal platform for attacking solid tumors in the CNS.

Methods Lentiviral vectors encoding an anti-CD19 CAR and secreted bridging proteins were created. The bridging proteins contained the CD19 extracellular domain, which is the target for the CAR, and anti-tumor antigen binding domains derived from antibodies (scFv and llama VH). The resulting anti-CD19 CAR T cells secrete the bridging proteins. These candidate cell therapeutics were evaluated for antigen binding and induction of antigen-specific cytotoxicity. An anti-CD19 CAR that secretes a CD19-anti-Her2 bridging protein has moved into development. Using the CD19-anti-Her2 bridging protein as a core module, we have begun evaluating a series of multi-antigen bridging proteins.

Results CAR-CD19 T cells that secrete bridging proteins have potent cytotoxic activity against single- and multi-antigen-positive cells. ALETA-002 is the lead candidate lentiviral vector construct encoding the anti-CD19 CAR domain and the CD19-anti-Her2 bridging protein, and has entered a GMP viral particle development campaign. This therapeutic will be systemically administered to Her2-positive breast cancer patients who are relapsing with CNS metastases. Next, multi-antigen bridging proteins encoding an anti-Her2 scFv and anti-B7H3, anti-B7H6 or anti-IL13Ra2 llama VH were assayed for potency. Lead candidates for development for the treatment of primary CNS malignancies were identified and are being manufactured at pilot-scale in 4-plasmid lentivirus production runs.

Conclusions The use of anti-CD19 CAR T cells that can expand off of the normal CD19-positive B cell pool enables tumor-antigen independent persistence, fitness and robust trafficking into the CNS. The use of small, modular bridging proteins allows us to leverage anti-CD19 CAR T cells and use these to attack solid tumor antigens that are present on CNS resident cancers and on CNS metastatic lesions. Novel cell therapeutics for the treatment of Her2-positive CNS metastases and heterogeneous primary CNS malignancies including glioblastoma and the pediatric gliomas have been developed.

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134 TUMOR-RESPONSIVE, MULTI-FUNCTIONAL GENETICALLY-ENGINEERED NATURAL KILLER CELLS FOR IMMUNOTHERAPY OF GliOBlastoma
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Background Despite aggressive treatments and care, the median survival for GBM patients is 14.6 months, which has only modestly improved over the past several decades, highlighting the need for new therapeutic approaches. NK cells, innate cytotoxic effectors, are showing potential for cancer immunotherapy for GBM.1–3 However, tumor antigen heterogeneity and a severely immunosuppressive tumor microenvironment (TME) have rendered GBM highly resistant to most single antigen-based NK monotherapies.1–6

Methods To overcome these challenges, our solution has been to develop a first multifunctional immunotherapy for GBM based on genetically-engineered NK cells bearing multiple simultaneous anti-tumor functions, including local tumor responsiveness and the ability to avoid antigen escape. The activity of these lentivirally-transduced multi-functional NK (E
Abstract 134 Figure 1 Multifunctional genetically-engineered NK cells for immunotherapy of GBM. (A) Schematic representation of transgene representing the complete multi-functional construct: tumor-responsive anti-CD73 scFv-secreting dual-specific CAR targeting NKG2DL and GD2. (B) Schematic representation of tumor-responsive anti-CD73 scFv secreting dual-specific CARs. (C) Flow cytometry data showing the purity of isolated peripheral blood-derived NK (pNK) cells (CD56+CD3-). (D) NKG2D expression on engineered pNK cells determined by flow cytometry after two rounds of lentiviral transduction. (E) Expression of anti-CD73 scFv and anti-GD2 scFv on pNK cells determined by flow cytometry after two rounds of lentiviral transduction. (F) In vitro cytotoxicity of pNK and E-pNK cells against different GBM43 at indicated E/T ratios over 4 h. (G) Degranulation (% CD107) and IFN-γ production of pNK and E-pNK cells (% IFN-γ) after 4 h coculture with GBM43 cells (E/T ratio, 5:1). (H) In vitro cytotoxicity of pNK and E-pNK (following aCD73 scFv cleavage) cells against GBM43 cells at indicated E/T ratios over 4 h. (I) CD73 activity of GBM43 cells after incubation with cleaved aCD73 scFv following cleavage from uPA-treated E-pNK cells. (J) Tumor growth of individual treatment groups, including PBS, pNK cells and E-pNK cells. Tumor size was determined by caliper measurements. (K) Average tumor weight of the mice in each treatment group after necropsy on day 28 post-start of treatment. (L) Changes in body weight of the mice in each group during the treatment period. *P < 0.05, **P < 0.01.

RESULTS

We have designed and synthesized a multifunctional CAR construct that expresses an anti-CD73 scFv which is cleavable by GBM-associated proteases, and a dual CAR redirected against ligands for NKG2D and GBM-associated GD2 receptors (figure 1A-B). We have isolated primary NK cells (figure 1C) and genetically manipulated them to express NKG2DL, anti-GD2 scFv and anti-CD73 scFv (figure 1D-E). E-pNK cells showed a significantly higher in vitro antitumor activity towards GBM43 targets, patient-derived GBM cells, including increased percentage of tumor killing, degranulation and IFN-γ production (figure 1F-G). E-pNK cells lacking the anti-CD73 scFv following uPA treatment displayed significantly decreased killing ability of target GBM43 cells after co-culture at E/T ratios of 2.5 and 5 for 4 h (figure 1H). In addition, after treatment with cleaved anti-CD73 scFv, GBM43 cells showed a significantly reduced ability to produce adenosine due to the inhibition of CD73 enzyme activity (figure 1I). Furthermore, E-pNK cells showed potent anti-GBM activity in subcutaneously GBM43 xenographs (figure 1J-L). In vivo-adoptively transferred E-pNK cells also showed superior intratumoral infiltration into GBM43 tumors when analyzed by IHC (data not shown).

Conclusions We have generated E-pNK cells showing improved antitumor activity against GBM through increased resistance to the immunosuppressive TME via adenosinergic CD73 blockade and the simultaneous ability to specifically target GBM cells via dual CARs. Based on these results, we are currently building the orthotopic GBM mouse model to further evaluate their in vivo therapeutic effects.

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Ethics Approval Primary human NK (pNK) cells used in this study were obtained using Purdue University’s Institutional Review Board (IRB)-approved consent forms (IRB-approved protocol #1804020540).

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TARGETING MET WITH CHIMERIC ANTIGEN RECEPTOR T CELLS IN HEPATOCELLULAR CARCINOMA

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Background Hepatocellular carcinoma (HCC) is the leading cause of cancer mortality worldwide. While HBV/HCV infection is the primary cause of HCC, overexpression of MET, the receptor of hepatocyte growth factor (HGF), occurs in 50% HCC patients, and is an indicator of poor prognosis. Although the multi-target MET tyrosine kinase inhibitor cabozantinib is FDA approved for treating advanced HCC, the long-term efficacy versus toxicity remains unknown. Our study is to develop specific MET-targeting chimeric antigen receptor T (CAR-T) cells for treating HCC with MET overexpression.

Methods Based on a well-established anti-MET monoclonal antibody, we synthesized and cloned the single-chain variable fragment (ScFv) sequence into two retroviral based 2nd generation CAR vectors (MET-CAR,CD28, and MET-CAR,CD28,CD137). A MET-CAR without CD3ζ domain (MET-CARA) served as a negative control. To produce MET-CAR-T cells, healthy PBMCs were stimulated with anti-CD3/CD28 antibodies in the presence of IL-7/IL-15 followed by transduction with MET-CAR viral particles. T cell transduction efficacy was...