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.

**REFERENCE**


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**TUMOR-RESPONSIVE, MULTI-FUNCTIONAL GENETICALLY-ENGINEERED NATURAL KILLER CELLS FOR IMMUNOTHERAPY OF Glioblastoma**

**Abstract**

**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 montotherapies.4–6

**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 NGK2DL1 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) NGK2DL1 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 tumor weight of the mice in each group during the treatment period. Note: the data shown in this study is for isolated pNK cells from one representative donor. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01.

pNK cells were evaluated against patient-derived GBM cells both in vitro and in vivo.

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 NGK2D and GBM-associated GD2 receptors (figure 1A-B). We have isolated primary NK cells (figure 1C) and genetically manipulated them to express NGK2D, 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 xenografts (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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136 TARGETING MET WITH CHIMERIC ANTIGEN RECEPTOR T CELLS IN HEPATOCELLULAR CARCINOMA

1Yuan Qin,1Anna Qin,1Anna Musket,1Joseph Lee,1Zhi Yao,2Giedre Krenciute,1Qian Xie*.1East Tennessee State University, Johnson City, TN, USA; 2St. Jude Children’s Research Hospital, Memphis, TN, USA

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.ζ and MET-CAR.4-1BB.ζ). 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...