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 the body 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 directed 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 NKG2D, 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-adaptively 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

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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,4-1BB,ζ). A MET-CAR without CD3ζ domain (MET-CARA) was 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

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determined using flow cytometry. HCC cell lines with variable MET expression from high/positive (MHCC97H, C3A, and JHHS) to MET low/negative (SNU398) were used to determine MET-specific CAR T cell specificity and effector function using MTS assay. We also collected media from the tumor-T cell co-cultures and determined IL-2 and IFNγ secretion using ELISA. Finally, real-time confocal imaging (24 h) was performed to record the progress of MET-CAR T cell mediated killing activity against MHCC97H/mCherry cells.

**Results** We show that both MET-CAR,CD28,ζ and MET-CAR,4-1BB,ζ -T cells significantly killed MHCC97H, C3A, and JHHS cells in antigen dependent manner. MET-CAR T cell killing is MET dependent as we observed no killing of MET-negative SNU398 cells. In addition, MET-CAR,4-1BB,ζ and MET-CAR,CD28,ζ -T cells secreted IL-2 and IFNγ when co-cultured with MHCC97H, C3A, JHHS cells, but not SNU398.

Confocal imaging studies showed that both MET-specific CAR T cells migrated toward MHCC97H/mCherry cells, formed aggregations, and induced tumor cell death, while MET-CARA T cells failed to do so.

**Conclusions** Here we demonstrate that MET-CAR,4-1BB,ζ and MET-CAR,CD28,ζ -T cells specifically recognize and kill MET-positive HCC cells in vitro. While animal studies are required to validate the efficacy in vivo, our study has produced a novel therapeutic CAR T cell target for treating malignant HCC and other type of cancers with MET overexpression.

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**Ethics Approval** The study was approved by East Tennessee State University’s Ethics Board, approval number #0619.3s.

**REFERENCE**