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 NGK22D 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) NGK2D 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 cytotoxicity of pNK and E-pNK cells against GBM43 cells (E/T ratio, 5:1). (H) In vitro cytotoxicity of pNK and E-pNK (following scCD73 scFv cleavage) cells against GBM43 cells at indicated E/T ratios over 4 h. (I) CD73 activity of GBM43 cells after incubation with cleaved scCD73 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 20 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 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 against ligands for NKG2D and GBM-associated GD2. (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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determined using flow cytometry. HCC cell lines with variable MET expression from high/positive (MHCC97H, C3A, and JHH5) 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 JHH5 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, JHH5 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.

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**GENOMICS OF MULTIPLE MYELOMA INFLUENCES THE EXPRESSION OF CAR T-CELL TARGETS**

1Christina Yu*, 2Brian Walker, 2David Rosdman, 3Yun Huang, 4Michel Sadelain, 2Fabiana Perna. 1Indiana University School of Medicine and Ohio State University, Indianapolis, IN, USA; 2Indiana University School of Medicine, Indianapolis, IN, USA; 3Indiana University School of Medicine and Regenstrief Institute, Indianapolis, IN, USA; 4Memorial Sloan Kettering Cancer Center, New York, NY, USA

**Background** Multiple Myeloma (MM) is an incurable disease, with a particularly poor prognosis for patients with refractory/relapsed MM or high-risk cytogenetics. Chimeric Antigen Receptor (CAR) T-cell therapy targeting BCMA can induce deep responses in highly pretreated RRMM; however, remissions are not sustained, and the majority of patients eventually relapse. We hypothesized that genomic determinants of MM play a role in dictating the expression of surface targets that can be of use for immune targeting.

**Methods** We analyzed the gene expression of 24 immunotherapeutic targets in a combined dataset of 1900 MM patients from three independent expression datasets obtained from the Multiple Myeloma Research Foundation CoMMpass study and Gene Expression Omnibus. Given that CAR T-cell therapy may be especially important for patients with high-risk myeloma, we defined the expression of each target in high-risk MM patients by stratifying patients based on several genomic features impacting prognosis. Additionally, we conducted a gene co-expression network analysis and identified 30 gene modules highly correlated with 16 cell surface targets from our panel, further suggesting that genetic determinants of MM may shape a targetable cell surfaceome. In order to determine whether targeting any of these candidate antigens might cause major toxicity to normal cells, we utilized several repositories providing protein data1 to annotate their expression in several normal cell types.

**Results** We determined that a number of genomic factors could stratify the 24 targets into three general groups: 1) targets that show consistent overexpression in high-risk patients: IGF1R, ITGB7, GPRC5D and CD70, and are thus suitable for most high-risk patients; 2) targets that are down-regulated in patients with high-risk genomic features: CD200, CD19, CD40, CD1D and IGKC, perhaps playing a role in cancer immune escape; and 3) targets associated with one specific genetic abnormality, i.e. t(4;14): FUT3, SLAMF7, CD56, CD138 and BCMA, thus of use for precision CAR therapy in this high-risk patient subset.

**Conclusions** Our work provides a means of target selection for precision CAR therapy, by considering both patient genomic backgrounds and cancer cell surface profiles. Furthermore, our results provide a roadmap for immunotherapy of MM by unbiasedly comparing the expression of top MM cell surface targets in patient data and normal cells and suggest that the genetic landscape of MM may predict the expression of specific targets for precision immunotherapy. The quest for novel MM targets for immunotherapies remains open, and CAR target discovery driven by specific genetic events remains an active area of investigation.

**REFERENCE**


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**IN VIVO LOCALIZATION OF GENETICALLY ENGINEERED NATURAL KILLER CELLS AGAINST GliOBLASTOMA USING PET IMAGING**

1Yeenee Yun*, 2Jiao Wang, 1Karen Pollok, 1Tony Sinn, 1Randy Brudakiewicz, 2Sandro Matosevic, 1Michael Veronesi, 1Indiana University School of Medicine, Indianapolis, IN, USA; 2Purdue University, West Lafayette, IN, USA

**Background** Glioblastoma (GBM) is a deadly brain malignancy with a dismal prognosis. While immunotherapy holds great promise for GBM treatment, most have failed due to a suppressive tumor microenvironment (TME). Antigen heterogeneity and adenosine signaling are two immunosuppressive mechanisms in GBM. The CD73-adenosine axis plays a multifaceted role in GBM pathogenesis and drives the dysfunction of NK cells in GBM TME.1,3 Our NKG2D-chimeric antigen receptor (CAR)-natural killer (NK) cells have shown anti-tumor activity when combined with CD73 blockade in vivo.2 To further extend the potency of these cells against GBM and address antigen heterogeneity in GBM, we combined the local blockade of CD73 with multi-antigen-targeting engineered NK cells. In order to improve treatment assessment, PET/MR imaging was employed to enable detailed, non-invasive assessment of tumor progression. Imaging assessment of adoptively-transferred CAR-NK cells was also developed to determine the fate of NK cell delivery to the tumor site over time.

**Methods** We generated multifunctional engineered NK (E-NK) cells that express an anti-CD73 scFv, which is cleavable by GBM-associated proteases, an NKG2D-CAR, as well as a GD2