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 cells 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-CARΔ 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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137 GENOMICS OF MULTIPLE MYELOMA INFLUENCES THE EXPRESSION OF CAR T-CELL TARGETS

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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 data[1] 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.

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138 IN VIVO LOCALIZATION OF GENETICALLY ENGINEERED NATURAL KILLER CELLS AGAINST GLIOBLASTOMA USING PET IMAGING

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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