

iPSC line to derive a multiplexed engineered, CAR-MICA/B iNK cell product candidate. Using a panel of tumor cell lines expressing MICA/B, CAR-MICA/B iNK cells displayed MICA specificity, resulting in enhanced cytokine production, degranulation, and cytotoxicity. Furthermore, in vivo NK cell cytotoxicity was evaluated using the B16-F10 melanoma cell line, engineered to express MICA. In this model, CAR-MICA/B iNK cells significantly reduced liver and lung metastases, compared to untreated controls, by 93% and 87% respectively.

**Conclusions** Ongoing work is focused on extending these pre-clinical studies to further support the clinical translation of an off-the-shelf, CAR-MICA/B iNK cell cancer immunotherapy with the potential to overcome solid tumor escape from NKG2D-mediated mechanisms of recognition and killing.

## REFERENCE

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## ENGINEERED T CELLS DIRECTED AT TUMORS WITH DEFINED ALLELIC LOSS

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**Background** Cell therapy, with all its promise as a powerful solid-tumor modality, is still hampered by the fundamental obstacle of cancer therapy: the acute shortage of truly tumor-specific targets. It is well known that an average tumor contains loss of heterozygosity (LOH) at an astonishing frequency: ~20% genome wide. These losses are irreversible and absolutely distinguish the cancer from normal cells.

**Methods** We describe a novel approach to cancer immunotherapy that draws on LOH as a large, so far untapped source of cancer targets. To exploit such allelic losses, we focus on polymorphic loci and target the remaining allelic product of a locus that has LOH. We engineer T cells with a modular signal-integration circuit designed to be activated only by tumor cells that have lost expression of one specific allele on their surface.

**Results** We use the HLA locus which undergoes LOH at a frequency of 13%, and the HLA-A\*02 allele specifically, as proof of concept. We present a large body of quantitative in vitro data, along with in vivo data, that support the use of a synthetic signal-integration circuit called Tmod as a cancer therapy. We also describe Tmod's mechanistic properties, including thorough structure/function analysis of its components.

**Conclusions** LOH is a rich source of new targets, provided a system of sufficient power can be devised to exploit them. Our Tmod signal integration system confers on engineered T cells the capacity to discriminate effectively between normal and tumor cells that contain specific allelic losses.

**Ethics Approval** The animal study was approved by Explora BioLabs' Ethics Board, protocol number EB17-010-059

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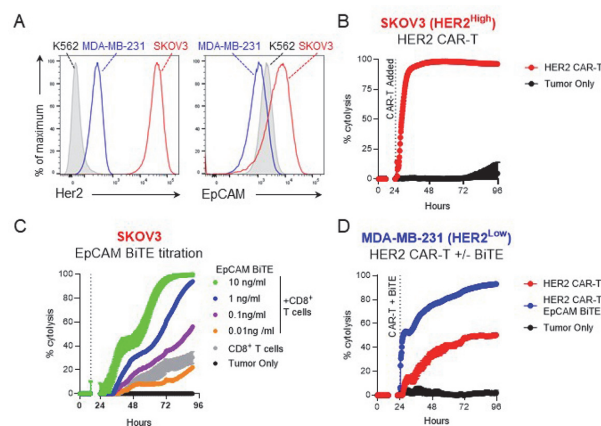
## MULTI-ANTIGEN TARGETING OF HETEROGENOUS SOLID TUMORS USING CAR T CELLS SECRETING BI-SPECIFIC T-CELL ENGAGERS

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**Background** Although CAR T cells have been shown to be effective and potent in treating several hematologic malignancies, engineered T-cell therapies have had limited success in addressing solid tumors. Unlike liquid tumors where uniformly expressed antigens are accessible and can be effectively targeted, tumor access and antigen heterogeneity are a significant barrier to the successful development of CAR-T cells in solid tumors.

**Methods** Here we demonstrate that the combination of a bi-specific T-cell engager (BiTE) targeting EpCAM with a CAR T cell targeting HER2 enhances the in vitro and in vivo anti-tumor activity against heterogenous solid tumors.

**Results** We observed a dose-dependent enhancement of cytolytic activity when EpCAM-specific BiTEs were titrated alongside 4D5-based HER2-specific CAR T cells against HER2low tumors, enhancing maximal cytolysis by two-fold compared to CAR T cells alone (figure 1). Moreover, the escape of HER2-low tumor cells in mixed heterogenous culture systems was circumvented by the combination of HER2-specific CAR T cells and EpCAM-specific BiTEs. The enhancement of efficacy was further demonstrated in an established HER2low MDA-MB-231 xenografts. HER2-specific CAR T cells were unable to contain Her2low tumors, whereas tumor growth was effectively controlled in mice receiving both EpCAM-specific BiTEs and HER2-specific CAR T cells.



**Abstract 116 Figure 1** EpCAM specific BiTEs supplement CAR-T efficacy in vitro (A) HER2 and EpCAM expression of SKOV3, MDA-MB-231, and K562 tumor cells was assessed by flow cytometry. (B) HER2 specific CAR-T rapidly targeted and lysed HER2High SKOV3 tumor cells as measured via xCelligence RTCA assay. (C) SKOV3 were co-cultured with untransduced CD8+ T cells and the indicated concentrations of EpCAM BiTE and specific cytolysis was assessed. (D) MDA-MB-231 (HER2low) tumor cells were co-cultured with HER2 CAR-T ± EpCAM BiTE and specific cytolysis was determined

**Conclusions** Collectively, these data demonstrate that multi-antigen targeting mediated by BiTEs and CARs extends overall anti-tumor efficacy in preclinical models of heterogenous solid