DEVELOPMENT OF T CELL-BASED IMMUNOTHERAPIES TO TARGET DORMANT DISSEMINATED BREAST CANCER CELLS

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Background A significant fraction of breast cancer survivors develop metastases years or even decades after initial diagnosis.1-3 Mounting evidence suggests these late recurrences arise from dormant disseminated tumor cells (DTCs).4-6 However, no therapy currently exists for targeting DTCs for the purpose of metastasis prevention. Immunotherapy represents a promising avenue to target dormant DTCs. Yet, a functional relationship between adaptive immunity and dormant DTCs has not been established.

Methods Here, we have utilized a bone marrow organotypic microvascular niche co-culture model and immunocompetent murine models of breast cancer dormancy to study the relationship between the adaptive immune response and dormant DTCs and to develop immunotherapies for the purpose of eliminating dormant DTCs and preventing breast cancer metastasis.

Results Our data suggest that breast cancer cells downregulate MHC class I antigen presentation upon dormancy induction, identifying one mechanism of immune evasion. Strikingly, outgrowing metastases re-express MHC I and presumably upregulate antigen presentation. These data suggest that MHC-dependent T cell-based immunotherapies may not effectively kill dormant DTCs, but that MHC-independent chimeric antigen receptor (CAR) T cells may be more applicable. Using the organotypic bone marrow microvascular niche co-culture system, we have shown that CAR T cells kill both proliferating and dormant tumor cells independent of tumor cell localization in the niche and independent of tumor cell cycle status. Further, we have established preclinical immunocompetent murine models of breast cancer dormancy to compare efficacy of engineered T cell receptor (TCR) and CAR T cells in eliminating dormant DTCs. From these models of breast cancer dormancy, we have found that CAR T cells eliminate both overt metastases and DTCs in the lung and bone marrow of mice. In contrast, preliminary data suggest that TCR T cells clear overt metastases but are less effective in clearing dormant disease, lending support that MHC I downregulation during dormancy may impact the efficacy of various T cell-based immunotherapies.

Conclusions Our findings identify CAR T cells as one potential immunotherapy to eradicate dormant disease, while simultaneously identifying both CAR and TCR T cells as effective treatments for the clearance of overt metastases. In sum, our findings lay the groundwork for developing adoptive cell therapies to eliminate dormant disease and prevent death from breast cancer metastasis.

REFERENCES
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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### Abstract 115

**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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### Abstract 116

**MULTI-ANTIGEN TARGETING OF HETEROGENEOUS 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 heterogeneous solid tumors.

**Results** We observed a dose-dependent enhancement of cytolysis when EpCAM-specific BiTEs were titrated alongside 4D5-based HER2-specific CAR T cells against HER2low tumors, enhancing maximal cytolyis by two-fold compared to CAR T cells alone (figure 1). Moreover, the escape of HER2-low tumor cells in mixed heterogeneous 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 heterogeneous solid