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 preclinical 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.

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115 ENGINEERED T CELLS DIRECTED AT TUMORS WITH DEFINED ALLELIC LOSS

Agi Hamburger*, Breanna DiAndreth, Jiajia Cui, Mark Daris, Melanie Munguia, Kiran Deshmukh, Jee-Young Mock, Grace Asuelime, Emily Lim, Michelle Kreke, Talar Tokatlian, Alexander Kamb*. *A2 Biotherapeutics, Inc., Agoura Hills, CA, USA*

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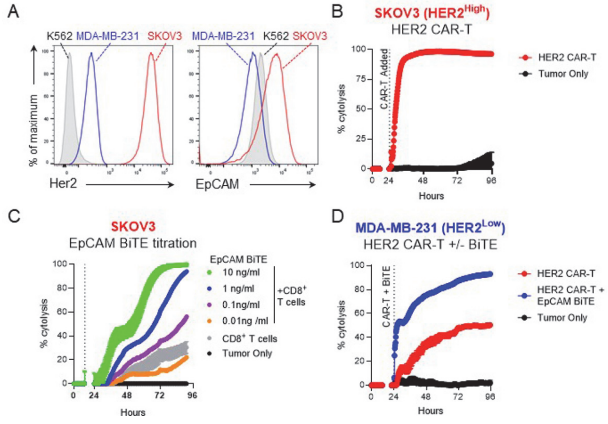
116 MULTI-ANTIGEN TARGETING OF HETEROGENOUS SOLID TUMORS USING CAR T CELLS SECRETING BI-SPECIFIC T-CELL ENGAGERS

Martin Hosking*, Bishwas Shrestha, Megan Boyett, Soheila Shirinbak, Angela Gentile, Eric Sung, Yijia Pan, Tom Lee, Jason ORourke, Dan Shoemaker, Cokey Nguyen, Bahram Valamehr. *Fate Therapeutics, Inc., San Diego, CA, USA*

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