combining the reduced affinity CAR with this exogenous control mechanism, we provide evidence that we can modulate and control CAR-mediated toxicity.

Ethics Approval All animal experiments were conducted in a facility accredited by the Association for Assessment of Laboratory Animal Care (AALAC) under Institutional Animal Care and Use Committee (IACUC) guidelines and appropriate animal research approval.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0112>

113

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

Erica Goddard*, Shivani Srivastava, Stanley Riddell, Cyrus Ghajar. Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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

1. Pan H, Gray R, et al. 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. *N Engl J Med* 2017;**377**, 1836–1846.
2. Karrison T, Ferguson D, Meier P. Dormancy of mammary carcinoma after mastectomy. *J Natl Cancer Inst*. 1999;**91**, 80–85.
3. Goss PE, & Chambers AF. Does tumour dormancy offer a therapeutic target? *Nat Rev Cancer* 2010;**10**, 871–877.
4. Aguirre-Ghisso JA. Models, mechanisms and clinical evidence for cancer dormancy. *Nat Rev Cancer* 2007;**7**, 834–846.
5. Klein CA. Framework models of tumor dormancy from patient-derived observations. *Curr Opin Genet Dev* 2011;**21**, 42–49.
6. Demicheli R, Abbattista A, Miceli R, Valagussa P, & Bonadonna G. Time distribution of the recurrence risk for breast cancer patients undergoing mastectomy: further support about the concept of tumor dormancy. *Breast Cancer Res Treat* 1996;**41**, 177–185.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0113>

114

PRECLINICAL DEVELOPMENT OF A NOVEL IPSC-DERIVED CAR-MICA/B NK CELL IMMUNOTHERAPY TO OVERCOME SOLID TUMOR ESCAPE FROM NKG2D-MEDIATED MECHANISMS OF RECOGNITION AND KILLING

¹John Goulding*, ¹Mochtar Pribadi, ¹Robert Blum, ¹Wen-I Yeh, ¹Yijia Pan, ¹Svetlana Gaidarova, ¹Chia-Wei Chang, ¹Hui-Yi Chu, ¹Ellen Liu, ¹Shohreh Sikaroodi, ²Lucas Ferrari de Andrade, ¹Lauren Fong, ¹Janel Huffman, ¹Tom Lee, ¹Ryan Bjordahl, ³Kai Wucherpfennig, ¹Bahram Valamehr. ¹Fate Therapeutics, San Diego, CA, USA; ²Icahn School of Medicine at Mount Sinai, New York, NY, USA; ³Dana-Farber Cancer Institute, Boston, MA, USA

Background MHC class I related proteins A (MICA) and B (MICB) are induced by cellular stress and transformation, and their expression has been reported for many cancer types. NKG2D, an activating receptor expressed on natural killer (NK) and T cells, targets the membrane-distal domains of MICA/B, activating a potent cytotoxic response. However, advanced cancer cells frequently evade immune cell recognition by proteolytic shedding of the $\alpha 1$ and $\alpha 2$ domains of MICA/B, which can significantly reduce NKG2D function and the cytolytic activity.

Methods Recent publications have shown that therapeutic antibodies targeting the membrane-proximal $\alpha 3$ domain inhibited MICA/B shedding, resulting in a substantial increase in the cell surface density of MICA/B and restoration of immune cell-mediated tumor immunity.¹ We have developed a novel chimeric antigen receptor (CAR) targeting the conserved $\alpha 3$ domain of MICA/B (CAR-MICA/B). Additionally, utilizing our proprietary induced pluripotent stem cell (iPSC) product platform, we have developed multiplexed engineered, iPSC-derived CAR-MICA/B NK (iNK) cells for off-the-shelf cancer immunotherapy.

Results A screen of CAR spacer and ScFv orientations in primary T cells delineated MICA-specific in vitro activation and cytotoxicity as well as in vivo tumor control against MICA+ cancer cells. The novel CAR-MICA/B design was used to compare efficacy against NKG2D CAR T cells, an alternative MICA/B targeting strategy. CAR-MICA/B T cells showed superior cytotoxicity against melanoma, breast cancer, renal cell carcinoma, and lung cancer lines in vitro compared to primary NKG2D CAR T cells ($p < 0.01$). Additionally, using an in vivo xenograft metastasis model, CAR-MICA/B T cells eliminated A2058 human melanoma metastases in the majority of the mice treated. In contrast, NKG2D CAR T cells were unable to control tumor growth or metastases. To translate CAR-MICA/B functionality into an off-the-shelf cancer immunotherapy, CAR-MICA/B was introduced into a clonal master engineered

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

1. Ferrari de Andrade L, Tay RE, Pan D, Luoma AM, Ito Y, Badrinath S, Tsoucas D, Franz B, May KF Jr, Harvey CJ, Kobold S, Pyrdol JW, Yoon C, Yuan GC, Hodi FS, Dranoff G, Wucherpfennig KW. Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell-driven tumor immunity. *Science* 2018 Mar 30;359(6383):1537–1542.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0114>

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

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0115>

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 O'Rourke, 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