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**


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, Jiอาja Cui, Mark Daris, Melanie Munguia, Kiran Deshmukh, Lee-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 HETEROGENEOUS SOLID TUMORS USING CAR T CELLS SECRETING BI-SPECIFIC T-CELL ENGAGERS**

Martin Hocking*, Bhishwa Shrestha, Megan Boyett, Soheila Shirinbak, Angela Gentile, Eric Sung, Yijia Pan, Tom Lee, Jason ORourke, Dan Shoemaker, Coley 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 heterogeneous 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 cytotoxicity 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 cytolyis was assessed. (D) MDA-MB-231 (HER2low) tumor cells were co-cultured with HER2 CAR-T ± EpCAM BiTE and specific cytolyis 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...
tumors. Fate Therapeutics is currently using its proprietary induced pluripotent stem cell (iPSC) product platform to generate iPSC-derived CAR T cells and iPSC-derived CAR NK cells that secrete BiTEs for the treatment of solid tumors.

**Ethics Approval** These studies were approved by Fate Therapeutics Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0116

---

**RAPID POINT-OF-CARE SUBCUTANEOUS CAR-T FROM BLOOD DRAW TO INJECTION IN 4 HOURS WITH MODIFIED LV ENCODING CARS AND SYNTHETIC DRIVER ELEMENTS ENABLES EFFICIENT CAR-T EXPANSION AND TUMOR REGRESSION**

Frederic Vigant*, Qun He, Wei Zhang, Hongliang Zong, Anirban Kundu, Ewa Jaruga-Killeen, Gregory Schreiber, Michelle Andraza, Alissa Kerner, Gregory Frost. Exuma Biotech Corporation, West Palm Beach, FL, USA

**Background** Adoptive cellular therapy with chimeric antigen receptor (CAR)-T cells has demonstrated remarkable clinical activity in a number of hematologic malignancies, but product chain of custody, individualized manufacturing, preparative chemotherapy, and patient management present technical and logistical hurdles to broader implementation.

**Methods** Lentiviral constructs for CARs (either CD19- or CD22-directed) co-expressed with a synthetic driver domain were identified from a >6 × 10 diversity combinatorial library of proliferative elements, transmembrane domains, leucine zippers, and an EGFR epitope screened for cellular expansion in a lymphoreplete model. Modified serum-free-lentiviral manufacturing process was developed to reduce complexity of CAR-T and to introduce CD3-activating elements into the viral envelope allowing activation and transduction of resting lymphocytes from peripheral blood.

**Results** Four-hour exposure of as little as 1 ml of blood to the CD3-directed CD19-targeted CAR encoding lentivirus followed by subcutaneous injection in NSG mice bearing CD19+/CD22+ Raji cells resulted in tumor regression (figure 1) and robust CAR-T cell expansion as determined by flow cytometry (figure 2) and qPCR (table 1), with peak levels >10,000 CAR-T cells/ml and less than three CAR copies per genome. In contrast, administration of the same products intravenously failed to support significant CAR-T expansion or control tumor growth (figure 3). Regression of established Raji tumors was also observed in NSG-(KbDb) (IA) animals following SC administration of CD19 or CD22 CARs with driver domains. CAR-T cells contracted in peripheral blood following tumor regression.

Regression of Raji tumor from the initial median volume of 151 mm3 throughout 40 days post subcutaneous administration of the LV transduced (at MOI 1 or 5) CD19-directed CAR T product (1M or 5M cells) in the NSG mice

**Conclusions** We conclude that through a synthetic subcutaneous lymph node approach with modified lentiviruses and driver domains, rPOC SC may enable CAR-T generation with reduced complexity, while maintaining the ability of CAR-T cells to expand, persist and exert anti-tumor activity.

**Ethics Approval** All animal studies were IACUC approved.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0117

---

**IL-6 IS CRITICAL FOR MEMORY RESPONSES ELICITED BY TH17 CELLS TO TUMORS**

Hannah Knochelmann*, Connor Dwyer, Aubrey Smith, Megan Wyatt, Guillermo Rangel Rivera, Gregory Lesinski, Zhili Li, Mark Rubinstein, Crystal Paulos. 1Medical University of South Carolina, Charleston, SC, USA; 2Emory University, Charleston, SC, USA; 3The Ohio State University, Columbus, Ohio, USA

**Background** Translation of novel T cell therapies is limited by cost and time-consuming protocols involving long-term T cell