

expression of activation markers (CD25, PD1, CD69) and proliferative capacity in response to anti-CD3/CD28 stimulation.

Results N/A

#### Abstract 110 Table 1

| Cell Type | T Cells | CD4+ T Cells | CD8+ T Cells | B Cells | NK Cells | Monocytes |
|-----------|---------|--------------|--------------|---------|----------|-----------|
| Mean      | 55.06%  | 33.47%       | 18.70%       | 6.88%   | 7.59%    | 18.10%    |
| %CV       | 14.52%  | 21.51%       | 32.68%       | 50.58%  | 38.47%   | 32.31%    |

**Conclusions** There was substantial variability (%CV 14.52%-50.58%, see table 1) in the percentage of each immune cell population across the donor pool, which would have effects on the relative success of downstream cell manufacturing. We are evaluating additional donors to identify specific sources of variability. Collectively, these data highlight the need for in-depth genotypic and phenotypic characterization of donor populations to ensure that the most robust material is selected for each type of cell therapy manufacturing.

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#### 111 HIGHLY EFFICIENT MULTIPLEXED BASE EDITING ENABLES DEVELOPMENT OF UNIVERSAL CD7-TARGETING CAR-T CELLS TO TREAT T-ALL

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**Background** Autologous CAR-T therapies have demonstrated remarkable efficacy in treating some hematologic cancers. However, generating bespoke cell therapies creates manufacturing challenges, inconsistent products, high cost of goods, and delays in treatment that are often incompatible with effective clinical management of patients. Strategies to create universally-compatible allogeneic CAR-T therapies have been developed as a solution to these challenges. Allogeneic CAR-Ts require mitigation of graft-versus-host-disease (GvHD), host rejection of CAR-Ts, and elimination of fratricide in instances where the target (e.g. CD7) is expressed on both malignant cells and healthy T-cells. Many allogeneic CAR-T approaches utilize DNA double strand break (DSB)-inducing nucleases to overcome these barriers. However, simultaneous induction of multiple DSBs results in unpredictable outcomes such as large-scale genomic rearrangements, megabase-scale deletions, and reduced cell proliferation. Here we leverage base editors (BEs), which are a novel class of gene editing reagents that enable programmable single-base changes in genomic DNA without forming DSBs, to create multiplex edited, fratricide resistant, allogeneic CAR-T cells with no detectable genomic aberrations.

**Methods** T-cell acute lymphoblastic leukemia (T-ALL) is a disease with high and consistent expression of CD7 on malignant T cells, making CD7-targeting CAR-Ts (7CAR-Ts) an attractive therapeutic agent. We developed a GMP-compatible process to create 7CAR-Ts at clinical scale by isolating T cells from healthy human donors and electroporating the cells with base editor reagents, followed by transduction with a lentiviral vector encoding a second generation anti-CD7 CAR. 7CAR-Ts were characterized for their potency and specificity in vitro and in xenograft tumor models.

**Results** Simultaneous base editing at four genomic loci resulted in 7CAR-Ts that are edited with 80–98% efficiency at each

target gene, with greatly diminished risk of GvHD, CAR-T rejection, fratricide, and immunosuppression. In contrast to nuclease editing, concurrent modification of four genomic loci using BEs produced no detectable genomic rearrangements, no observable change in cell expansion, and no activation of the DNA damage-induced p53 pathway. Base edited 7CAR-Ts demonstrate robust antigen-dependent cytokine release, potent in vitro cytotoxicity, and dose-dependent in vivo tumor control.

**Conclusions** Taken together, our approach addresses limitations in CAR-T manufacturing and demonstrates that multiplexed base editing is a feasible strategy for generating universally-compatible, fratricide-resistant 7CAR-T cells, which we are advancing towards clinical development for the treatment of T-ALL. More generally, this program demonstrates the potential for base editing to create highly-engineered cell therapies featuring at least four simultaneous edits which can confer a wide range of desirable therapeutic attributes.

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#### 112 RATIONAL DESIGN OF CHIMERIC ANTIGEN RECEPTOR T CELLS AGAINST GLYPICAN 3 DECOUPLES TOXICITY FROM THERAPEUTIC EFFICACY

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**Background** Chimeric antigen receptor (CAR)-T therapy has yielded impressive clinical results in hematological malignancies and it is a promising approach for solid tumor treatment. However, toxicity, including on-target off-tumor antigen binding, is a concern hampering its broader use.

**Methods** In selecting a lead CAR-T candidate against the oncofetal antigen glypican 3 (GPC3), we compared CAR bearing a low and high affinity single-chain variable fragment (scFv) binding to the same epitope and cross-reactive with murine GPC3. We characterized low and high affinity CAR-T cells immunophenotype and effector function in vitro, followed by in vivo efficacy and safety studies in hepatocellular carcinoma (HCC) xenograft models.

**Results** Compared to the high-affinity construct, the low-affinity CAR maintained cytotoxic function but did not show in vivo toxicity. High-affinity CAR-induced toxicity was caused by on-target off-tumor binding, based on the evidence that high-affinity but not low-affinity CAR, were toxic in non-tumor bearing mice and accumulated in organs with low expression of GPC3. To add another layer of safety, we developed a mean to target and eliminate CAR-T cells using anti-TNF $\alpha$  antibody therapy post-CAR-T infusion. This antibody functioned by eliminating early antigen-activated CAR-T cells, but not all CAR-T cells, allowing a margin where the toxic response could be effectively decoupled from anti-tumor efficacy.

**Conclusions** Selecting a domain with higher off-rate improved the quality of the CAR-T cells by maintaining cytotoxic function while reducing cytokine production and activation upon antigen engagement. By exploring additional traits of the CAR-T cells post-activation, we further identified a mechanism whereby we could use approved therapeutics and apply them as an exogenous kill switch that would eliminate early activated CAR-T following antigen engagement in vivo. By

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.

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#### 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.<sup>1–3</sup> Mounting evidence suggests these late recurrences arise from dormant disseminated tumor cells (DTCs).<sup>4–6</sup> 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.

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#### 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

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**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.<sup>1</sup> 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