

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

Jason Gehrke\*, Aaron Edwards, Ryan Murray, Angelica Messana, Lindsey Coholan, Henry Poulin, Melissa Le, Alden Ladd, Mark Naniang, Faith Musenge, Adam Camblin, Yeh-Chuin Poh, Sarah Smith, Giuseppe Ciaramella. *Beam Therapeutics, Cambridge, MA, USA*

**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

Letizia Giardino\*, Ryan Gilbreth, Cui Chen, Erin Sult, Noel Monks, Ravinder Tammali, Jon Chesebrough, Kevin Schifferli, Nina Chu, Jessica Tong, Lori Clarke, Christina Gesse, Xiao-Tao Yao, Karma DaCosta, Kapil Vashisht, Gordon Moody. *AstrZeneca, gaithersburg, MD, USA*

**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