or hMSLNpos human mesothelioma cell line, MSTO-211H, or stimulated with anti-CD3/anti-CD28 antibodies in vitro for 8 days. Distinct cell populations in MCY-M11 were evaluated for kinetics and duration of CAR expression, differentiation, activation, exhaustion, and their ability to secrete various immunomodulatory molecules during in vitro stimulation. Antigen-specific proliferation and cytotoxicity of MCY-M11 against hMSLNpos tumor cells as well as their ability to mount long-term antitumor immunity through epitope spreading mechanisms were studied.

Results Individual cell populations in MCY-M11 exhibited a consistent but transient Meso-CAR expression persisting for about 7 days. Cell subsets in MCY-M11 acquired early signs of activation and differentiation within 18–24 hours post-culture, but only attained full activation and lineage-specific differentiation upon specific response to hMSLNpos tumor cells. hMSLN antigen experienced MCY-M11 retained significant fractions of Naïve and Central Memory T cells and increased percentage of Effector Memory T cells along with increased expression of CD62L, CD27, and chemokine receptors (CCR5, CCR7, and CXCR3). MCY-M11 exhibited strong antigen-specific cytotoxicity against hMSLNpos tumor cells with corresponding increase in activation and proliferation of CD4+ and CD8+ T cell subsets and displayed low or no acquisition of known exhaustion markers. NK cells also exhibited a functionally superior molecular signature exhibiting increased levels of NKG2D, NKp44, NKp46, FAS, and TRAIL. The Monocytes and B cells in MCY-M11 also acquired an activated, differentiated, and mature phenotype, expressing molecules required for antigen presentation (HLA-DR, HLA-ABC, and CD205) and T cell co-stimulation (CD80 and CD86) to mount a strong antitumor response. These phenotypic changes in cell subsets of MCY-M11 transpired with simultaneous secretion of potent immunostimulatory molecules and chemokines facilitating an extended antitumor response through epitope spreading.

Conclusions We demonstrated that MCY-M11 is a unique cell product possessing a complete built-in immune cellular machinery with favorable phenotype and enhanced functions specialized in mediating an effective and long-term antitumor response.

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109 DOMINANT-NEGATIVE TGFβ RECEPTOR 2 ENHANCES GPC3-TARGETING CAR-T CELL EFFICACY AGAINST HEPATOCELLULAR CARCINOMA

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Background Chimeric antigen receptors (CARs) are engineered synthetic receptors that reprogram T cell specificity and function against a given antigen. Autologous CAR-T cell therapy has demonstrated potent efficacy against various hematological malignancies, but has yielded limited success against solid cancers. MEDI7028 is a CAR that targets oncofetal antigen glypican-3 (GPC3), which is expressed in 70–90% of hepatocellular carcinoma (HCC), but not in normal liver tissue. Transforming growth factor β (TGFβ) secretion is increased in advanced HCC, which creates an immunosuppressive milieu and facilitates cancer progression and poor prognosis. We tested whether the anti-tumor efficacy of a GPC3 CAR-T can be enhanced with the co-expression of dominant-negative TGFβRII (TGFβRIIDN).

Methods Primary human T cells were lentivirally transduced to express GPC3 CAR both with and without TGFβRIIDN. Western blot and flow cytometry were performed on purified CAR-T cells to assess modulation of pathways and immune phenotypes driven by TGFβ in vitro. A xenograft model of human HCC cell line overexpressing TGFβ in immunodeficient mice was used to investigate the in vivo efficacy of TGFβRIIDN armored and unarmored CAR-T. Tumor infiltrating lymphocyte populations were analyzed by flow cytometry while serum cytokine levels were quantified with ELISA.

Results Armoring GPC3 CAR-T with TGFβRIIDN nearly abolished phospho-SMAD2/3 expression upon exposure to recombinant human TGFβ in vitro, indicating that the TGFβ signaling axis was successfully blocked by expression of the dominant-negative receptor. Additionally, expression of TGFβRIIDN suppressed TGFβ-driven CD103 upregulation, further demonstrating attenuation of the pathway by this armoring strategy. In vivo, the TGFβRIIDN armored CAR-T achieved superior tumor regression and delayed tumor regrowth compared to the unarmored CAR-T. The armored CAR-T cells infiltrated HCC tumors more abundantly than their unarmored counterparts, and were phenotypically less exhausted and less differentiated. In line with these observations, we detected significantly more interferon gamma (IFNγ) at peak response and decreased alpha-fetoprotein in the serum of mice treated with armored cells compared to mice receiving unarmored CAR-T, demonstrating in vivo functional superiority of TGFβRIIDN armored CAR-T therapy.

Conclusions Armoring GPC3 CAR-T with TGFβRIIDN abrogates the signaling of TGFβ in vitro and enhances the antitumor efficacy of GPC3 CAR-T against TGFβ-expressing HCC tumors in vivo, proving TGFβRIIDN to be an effective armoring strategy against TGFβ-expressing solid malignancies in preclinical models.

Ethics Approval The study was approved by AstraZeneca's Ethics Board and Institutional Animal Care and Use Committee (IACUC).

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110 IN-DEPTH CHARACTERIZATION OF VARIABILITY IN APHERESIS COLLECTIONS FROM NORMAL DONOR POPULATIONS FOR ALLOGENEIC CELL THERAPY

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Background The success of autologous CAR-T cell therapies has revolutionized and accelerated development in the cell therapy field. However, the requirement for patient-specific starting material for these therapies remains an impediment to establishing availability for all patients who could benefit, highlighting the need for a highly characterized normal donor pool to generate allogeneic cell therapy material.

Methods We have established a network of >2800 normal donors that have been genotyped at the HLA loci (6 digits) and stratified by reactivity to common human viruses, such as cytomegalovirus (CMV) and Epstein Barr Virus (EBV). Furthermore, cell collections from 35 randomly selected donors have been screened by flow cytometry for major immune cell subsets, including T cells, B cells, NK cells, and monocytes. The T cell compartment was further characterized by

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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-TNFα 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