



Universal chimeric antigen receptor-T cells: An opening era for T-cell malignancies

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Universal chimeric antigen receptor-T cells (CAR-T) refer to a type of CAR T-cell therapy designed for use across multiple patients without the need for individualized manufacturing. The advantage of universal CAR-T therapy lies in its off-the-shelf availability, eliminating the need for personalized manufacturing for each patient. Traditional CAR-T therapies require the extraction of a patient's T cells, which are then genetically modified and expanded in the laboratory before being infused back into the same patient. The relatively high cost associated with CAR-T therapy, attributed to its individualized and complex manufacturing process, hinders its widespread adoption and accessibility to the broader population. Furthermore, traditional CAR-T cell production involves a series of intricate steps, with the failure of any one process resulting in the overall failure of CAR-T cell preparation. In contrast, universal CAR-T therapies are derived from healthy donor T cells that have been genetically modified to express the CAR. These modified cells can be manufactured in large quantities and frozen for future use, making them readily available for treatment when needed. This approach allows for a more streamlined and cost-effective process, potentially increasing accessibility to CAR-T therapy for a larger number of patients.

Another advantage of universal CAR-T is the potential to address certain limitations of autologous CAR-T therapy. Autologous CAR-T therapy can be challenging for patients with heavily treated or rapidly progressing cancers, as obtaining viable T cells from these individuals may be difficult. Universal CAR-T could provide an alternative treatment option for such patients, as it is not dependent on their own T cells. This advantage makes universal CAR-T particularly suitable for T-cell-derived malignancies.

However, it is important to note that universal CAR-T therapy is still a developing field, and there are challenges to overcome, such as the risk of graft-versus-host disease (GVHD) due to the use of donor T cells. In recent years, comprehensive research on universal CAR-T cells has been conducted worldwide. To avoid potential GVHD caused by allogeneic T cells, T-cell gene editing has become essential. The three representative gene editing technologies commonly employed for gene knockout (i.e., Zinc Finger Nucleases [ZFNs], Transcription Activator-Like Effector Nucleases [TALENs], and Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 [CRISPR/Cas9]) are all based on inducing DNA double-strand breaks at target genomic loci, thereby activating cellular repair mechanisms. DNA double-strand break repair mechanisms in cells include random insertions, deletions through non-homologous end joining (NHEJ), and homology-directed repair (HDR) requiring a homologous template. However, this strategy is not without its inherent risk. For instance, ALLO-501A, developed by Allogene employs TALENs to disrupt the genes encoding T-cell receptor (TCR) and CD52 in T cells obtained from healthy donors, with the aim of mitigating the risk of GVHD. As early as October 2021, the FDA halted all clinical trials conducted by Allogene due to the occurrence of chromosomal abnormalities (which possess potential carcinogenic risks) in a patient who underwent treatment with ALLO-501A.¹ Although the FDA lifted the ban three months later, the potential hazards associated with gene editing should not be underestimated.

CRISPR-mediated DNA base editing (BE) is a revolutionary gene editing technology that allows precise changes to individual DNA bases without causing double-strand breaks. This technology offers several distinct advantages over traditional gene editing tools like TALENs and standard CRISPR-Cas9. The BE systems typically consist of two main components—a catalytically impaired Cas9 protein (nickase or dCas9) and a deaminase

enzyme that can change one DNA base pair to another. When guided by a gRNA to a specific target sequence within the genome, the Cas9 nickase creates a single-strand break in the DNA, which activates the cellular DNA repair machinery. During repair, the deaminase enzyme within the base editor catalyzes a precise chemical change to the target DNA base, converting it to another base, such as cytosine (C) to thymine (T). The end result is a targeted and specific alteration of a single DNA base pair without introducing double-strand breaks. The BE technology allows for highly precise and specific edits at the single-nucleotide level. This precision reduces the risk of off-target effects seen in traditional gene editing tools and induces minimal DNA damage, significantly reducing the potential for p53-mediated DNA damage response, chromosomal deletions, or genomic rearrangements. Despite these advantages, it's important to note that CRISPR base editing has its limitations, including the range of targetable sequences, and the potential for off-target effects, albeit at a lower rate than traditional CRISPR-Cas9. Additionally, base editing may lead to severe bystander effects, resulting in multiple unnecessary base conversions within the editing window. Nevertheless, BE technology has opened up significant possibilities for relatively safe gene editing of CAR-T cells.²

CD7 is a transmembrane glycoprotein expressed by T and NK cells and their precursors. It is also expressed in over 95% of lymphoblastic T-cell leukemias and lymphomas, as well as in a subset of peripheral T-cell lymphomas. Notably, CD7 is not expressed on some normal T cells, and its absence does not significantly impact the development, maturation, or function of T cells. Due to these characteristics, CD7 is considered an attractive target for CAR-T cell therapy in T-lineage malignancies. However, CAR expression in T lymphocytes leads to fratricide due to the presence of CD7 in the T cells themselves, resulting in a delay in the development of CD7 CAR-T cell therapies. Investigators have employed CD7 disruption techniques, including gene and base editing using CRISPR/Cas9, and have also sequestered CD7 internally using a protein expression blocker to create fratricide-resistant CD7 CAR T cells. Instead of genetically engineering T cells to render them CD7 negative, naturally occurring CD7 negative T cells might also serve as a promising cell source for generating CD7 CAR-T cells. Some of these approaches are currently under investigation in early-phase clinical trials, with encouraging results (#NCT04004637, #ChiCTR2000034762, #NCT03690011, #NCT04572308).³

In a recent clinical study, Chiesa et al. employed a single-dose combination of three sgRNAs targeting TRBC, CD52, and CD7, along with mRNA encoding a codon-optimized cytidine base editor (coBE), to edit the DNA of healthy donor T cells.⁴ Notably, CD7 allogeneic CAR-T cells (BE-CAR7) were prepared with a Prodigy device enabling a largely automated process. Moreover, the sgRNA targeting TRBC enabled simultaneous knockout of two homologous gene segments, TRBC1 and TRBC2, further enhancing the efficiency of TCRαβ knockout to avoid GVHD. The inactivation of CD7 expression on BE-CAR7 by base editor was to avoid CAR-T fratricide, a phenomenon typically occurs when CAR-T cells are designed to recognize specific antigens also expressed on T cells, leading to a mutual attack and cell death which can significantly reduce the effectiveness of CAR-T cell therapy and limit its therapeutic potential. The CD52 expressed on BE-CAR7 was inhibited in the same way to evade lymphodepletion therapy before CAR-T infusion with alemtuzumab, a monoclonal antibody targeting CD52 (Figure 1). As for safety concerns for multiple base editing procedure, chromosomal

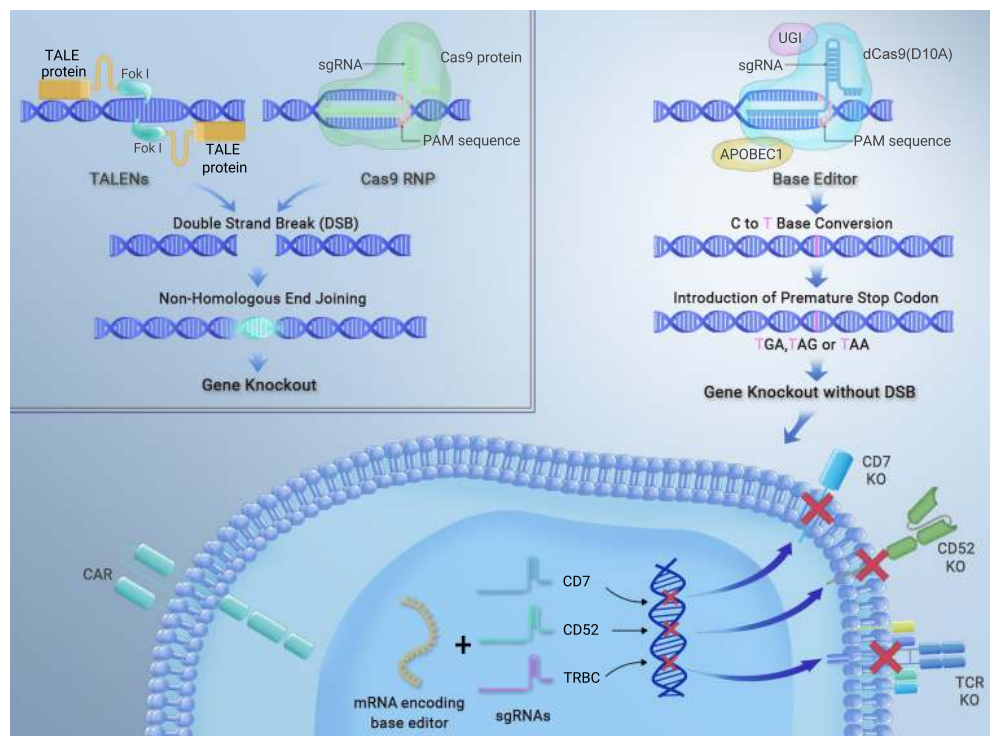


Figure 1. Gene editing tools used in making CD7-targeted universal CAR-T cells Upper left: Mechanisms of gene editing technology of TALENs and CRISPR/Cas9 to disrupt a gene; Right: Generation of CD7-targeted universal CAR-T cells (BE-CAR7) by Base Editor.

sustained presence and functionality of CAR-T cells. An interesting observation from a donor-derived CD7 CAR-T phase I trial by Pan et al. suggested that despite the depletion of patients' CD7-positive normal T cells, CD7-negative T and Natural Killer cells were still able to expand, but whether this CD7-negative cell population could exert normal immune protective function remained undetermined.⁵ Another approach to tackle the T-cell depletion may involve the incorporation of safety switches into the CD7 CAR-T cell construct, thus creating a controllable therapeutic window for tumor eradication and subsequent immune reconstitution.

Second, the current results from CD7 universal CAR-T trials are still preliminary in terms of cohort size and follow-up period. The long-term safety profiles of genetically modified cell products are still unknown. It is of note that

variations were not detected in the generated cells during karyotype analysis, and translocation efficiency was found to be <0.1% by droplet digital PCR. Flow cytometry analysis demonstrated knockout efficiencies of TCR, CD7, and CD52 exceeding 98%. These findings validate the highly efficient and safe nature of base editing.

Subsequently, Chiesa et al. conducted a phase I clinical trial, utilizing BE-CAR7 cells, to treat relapsed/refractory pediatric acute lymphoblastic leukemia (ALL) patients. Based on the treatment data collected from the three pediatric patients who received BE-CAR7 cell infusions (with the longest follow-up period being 8 months and the shortest being 28 days), all three patients achieved complete remission (CR) within 28 days, without experiencing graft-versus-host immune reactions. However, all three patients developed cytokine releasing syndrome (CRS) within 28 days, with two patients experiencing Grade 1 immune effector cell-associated neurotoxicity syndrome (ICANS). Manifestations of CRS, such as fever and rash, developed within the first week of treatment in each of the patients. One patient with high disease burden developed biphasic cytokine release syndrome, with overlapping sepsis and fungal lung disease which need intensive care. Additionally, the immune deficiencies resulting from BE-CAR7 infusion should not be overlooked, as all patients experienced continuing lymphopenia and two patients exhibited viral reactivations including cytomegalovirus (CMV), while another patient died with progressive lung complications from overlapping fungal infection.

While Chiesa et al.'s study successfully achieved industrial-scale production of CD7 universal CAR-T cells through base editing and provided initial evidence of their effectiveness in treating relapsed/refractory T cell ALL patients, further research is required to address the several challenges faced by CD7 universal CAR-T.

First, unlike well-established CD19 CAR-T therapy in B-cell malignancies in which treatment-related lymphopenia could be readily compensated by supplementation of γ -globulin to alleviate humoral defect due to eradication of B-cells, severe immune deficiencies resulting from lymphocyte depletion and the dual impact of CD7 targeted CAR-T cell infusion have led to significant concomitant infections following tumor clearance. Although the incidence of viral infections varies across reported CD7 CAR-T trials, the currently used methods to mitigate prolonged cytopenia usually include subsequent hematopoietic stem cell transplantation following CD7 CAR-T infusion, highlighting the dilemma faced by CD7 CAR-T cells, namely the conflicting needs for immune environment restoration in patients and the

different combinations of genes were knock-out or inactivated through various gene editing technology to generate universal CAR-T cells across trials. Even with the advanced BE system, careful experimental design to identify highly efficient sgRNAs for editing and thorough potential off-target effects screening are essential for simultaneous multiple gene inactivation. In the BE-CAR7 study, this genotoxicity concern was partially mitigated by limited timespan of CAR-T expansion *in vivo* to avoid long-term engraftment. But potential risks will arise when such products are applied in other T-cell malignancies such as T-cell lymphoma, which may require more persistent CAR-T expansion and longer time to achieve CR.

In conclusion, the BE-CAR7 study demonstrates the utility of base editing as a novel gene editing method for the industrial-scale production of universal CAR-T cells, following in the footsteps of TALENs, CRISPR/Cas9, and Sleeping Beauty transposons. This paves the way to an exciting future of broad applicable universal CAR-T cells with both efficacy and safety.

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DECLARATION OF INTERESTS

The authors declare no competing interests.