

Life Sciences and Medicine

Special Topic: Gene Editing towards Translation

Harnessing the power of gene-editing to develop the next generation of CAR-T cells

Bing Du*, Jiqin Zhang & Mingyao Liu*

Shanghai Frontiers Science Center of Genome Editing and Cell Therapy, Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai 200241, China

*Corresponding authors (emails: bdu@bio.ecnu.edu.cn (Bing Du); myliu@bio.ecnu.edu.cn (Mingyao Liu))

Received 6 December 2022; Revised 3 March 2023; Accepted 5 April 2023; Published online 1 August 2023

The manufacturing workflow of CAR-T cells makes it easy to be further modified by gene editing strategies. The rapid development of gene editing technology not only unveiled the mystery of T cells, but also provided a huge space for the optimization and development of the CAR-T cells. Here, we review the crucial role of gene editing in improving the safety, efficacy and accessibility of CAR-T cells in clinic.

Owing to the continuous advances in gene editing technology, the CRISPR-Cas9 as well as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) mediated genome modification has been widely used in both basic research and clinical applications, especially in genetic disorders and cancers [1,2]. Meanwhile, chimeric antigen receptor (CAR) T cell therapy has shown dramatic efficacy in the treatment of hematological cancers and been approved for commercial use [3]. However, the application of CAR-T cell therapy in treating solid tumors was restricted by unaffordable price and poor efficacy. The rapid and versatile approach for genomic modification by gene editing technology makes it possible to extend the application of T cell therapy through optimizing the manufacturing process and clinical efficacy [4]. Thus, different approaches have been employed in the identification of the key regulators in T cells, generating enhanced CAR-T cells, developing allogeneic universal CAR-T cells and revolutionary optimization to manufacturing techniques and functions of CAR-T cells (Figure 1).

Functional screening of key regulators in T cells using CRISPR-Cas9. Limited persistence, proliferation and cytotoxicity of CAR-T cells restrict the development of effective CAR-T therapies to solid tumor as well as hematological cancer. Therefore, multiple genome-wide CRISPR knock-out screens have been approached to identify genes involved in T cell dysfunction. Through unbiased genetic screening that mimics tumor microenvironment (TME) under several immunosuppressive conditions, RASA2, a RAS GTPase-activating protein (RasGAP) was demonstrated to be a promising target, which can enhance the persistence and effective function of T cells in treating cancers [5]. However, loss-of-function screens based on CRISPR are limited to exploring negative regulators of T cell functions [6,7]. The gain-of-function screening can be also mediated by a dead-guide RNA (dgRNA)-based CRISPR to identify proline dehydrogenase 2 (PRODH2/Prodh2) as the positive regulator of T cell functions [8]. Furthermore, barcoded

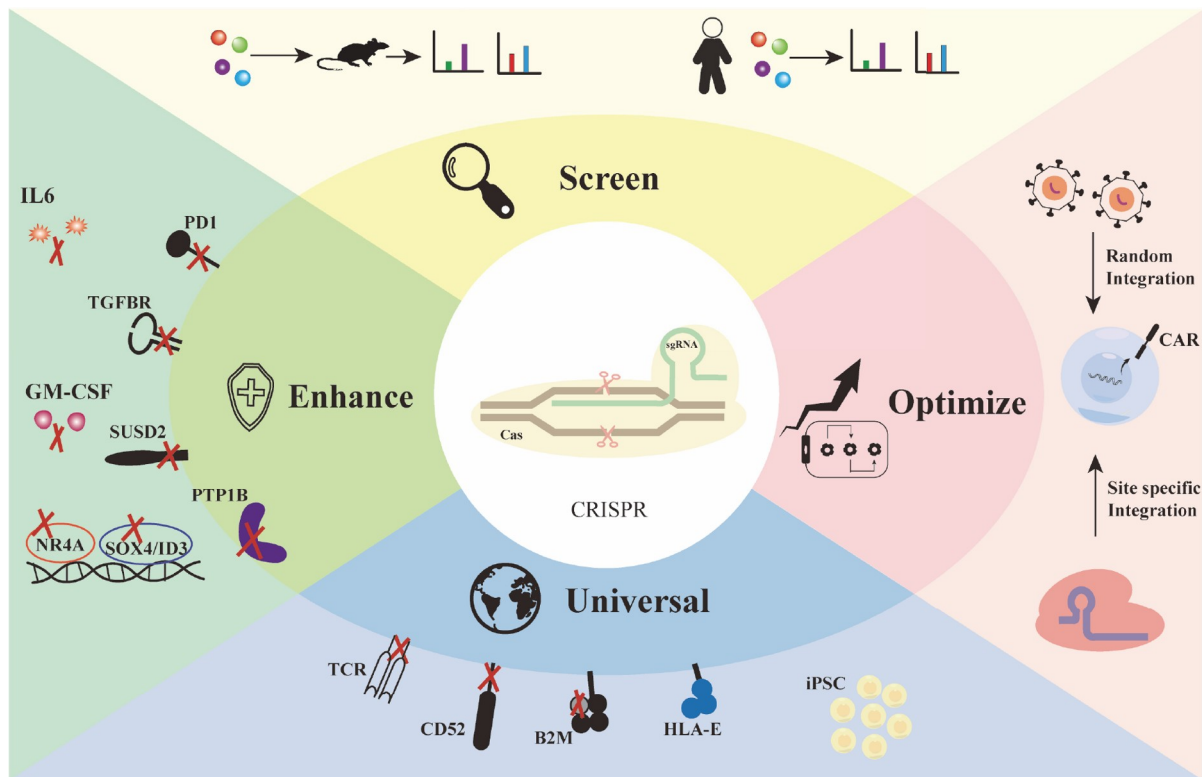


Figure 1 The application of CRISPR-Cas9 system in CAR-T therapy.

human open reading frames (ORFs) overexpression was used to identify positive regulators of T cell functions such as lymphotoxin- β receptor (LTBR) which had great potential in future cancer-agnostic therapies [9]. Thus, the functional screening of key regulators provides a number of strategies for improving next-generation T cell therapies through the loss-of-function as well as gain-of-function screens and induction of synthetic cell programs.

Enhancing the potential of CAR-T cells by endogenous gene modification. The *in vitro* manufacturing process makes it easy to influence the gene expression of CAR-T cells through gene editing tools. As the most popular immune checkpoint, the inhibitory effects of PD-1 have been disrupted by CRISPR-Cas9 for a long time which result in enhanced antitumor activity in both preclinical and clinical studies [10–12]. In comparison, the genetic depletion of mitogen-activated protein kinase 1 (MAP4K1) by CRISPR-Cas9 was demonstrated to be more effective than genetic knockout of PD-1 in CAR-T cells [13]. Meanwhile, T cell-specific protein tyrosine phosphatase 1B (PTP1B) deletion repressed tumor growth and strengthened the response to PD-1 blockade [14]. Deletion of sushi domain containing 2 (SUSD2) improves antitumor efficacy of CAR-T cells as well [15]. CRISPR-edited GM-CSF knockout in addition to autonomous IL6/IL1 blockades could minimize both the efficiency and safety issues such as GM-CSF-associated toxicity in CAR-T therapy [16]. In addition, the CAR gene was successfully inserted into the TCR α subunit constant gene (TRAC) by the CRISPR/Cas system combined with HDR, thus blocking the expression of endogenous TCR expression [17]. Furthermore, IL-12P70 was integrated into programmed cell death protein 1 (PDCD1) gene or IL2R α , which leads to a transient, antigen concentration-dependent secretion of IL-12P70, increases the

cytotoxicity of CAR-T cells, and prolongs survival of tumor-bearing mice [18]. Taken together, with the assistance of gene editing technology, efficacy of CAR-T cells could be enhanced significantly to get better therapeutic outcomes.

Generation of allogeneic universal CAR-T cells by gene editing. The application of personalized CAR-T cells was restricted by significant clinical and logistical barriers [19]. Therefore, an “off the shelf” allogeneic CAR-T therapy is an attractive solution to improve the accessibility of CAR-T cells to all patients. Gene editing technologies have yielded strategies to control GVHD risk by efficiently eliminating T cell receptor (TCR) expression, and have opened up new techniques to render allogeneic CAR-T cells invisible (or at least minimally visible) to the host immune system. Simultaneously disrupting TCR and CD52 by TALENs in T cells, the “off-the-shelf” CAR-T cells (UCART19) showed *in vivo* expansion and anti-leukaemic activity with a controllable safety in patients with relapsed or refractory B cell acute lymphoblastic leukemia [20]. Similarly, CD19/CD22-targeting CAR-T cells (CTA101) with TRAC region and CD52 gene depletion using CRISPR/Cas9 system were also evaluated in clinical treatment of the patients with relapsed/refractory acute lymphoblastic leukemia (r/r ALL) [21]. The human leukocyte antigen (HLA)-I-deficient T-cell generated by disrupting B2M loci is another way to develop immune-evasive universal CAR-T cells, whereas the depletion of HLA-I molecules will cause the activation of NK cells. Thus, a NK-inhibitor should be introduced to evade NK cell attacks. Combined with recombinant adeno-associated virus 6 (AAV6) and multiplex TALENs treatments, universal CAR-T cells were constructed by inserting a CAR and HLA-E, a non-polymorphic NK inhibitor into the B2M and TRAC loci, respectively. These allogeneic CAR-T cells were shown to evade attack by NK cells and alloresponsive T-cells and to extend their persistence and antitumor activity in the condition of NK cell induced cytotoxic levels *in vivo* and *in vitro* [22]. After genetically depletion of CD7, HLA-II and TCR, in co-expression with the common cytokine receptor γ chain and NK cell inhibitor (NKi), the CD7-targeting allogeneic CAR-T cells were demonstrated to have encouraging safety and efficacy profiles for relapsed/refractory CD7-positive hematological malignancies [23]. And the gene editing strategy used in T cells could also be extended to induced pluripotent stem cells (iPSCs) which could be developed into different kinds of universal cells for particular clinical applications [24]. Although the generation of allogeneic universal CAR-T cells still faces many challenges, we believe that the next generation of universal CAR-T cells will benefit a broader range of patients after improving the therapeutic outcomes and allowing them to be used on a large-scale in multiple malignancies.

Revolutionary developments optimize the manufacturing process and function of CAR-T cells. Even though 9 kinds of CAR-T products have been approved to enter the market, the viral vector is still the only method for manufacturing commercialized CAR-T cells. However, viral production is currently very costly up to 40% of the total cost of CAR-T cells, and the random integration of viral DNA in the chromosomes is also a subject of concern. Therefore, the non-viral and specifically targeted manufacturing process of CAR-T cells is urgently needed. Whereas, homology-directed repair (HDR) is relatively rare and DSBs are mainly repaired by non-homologous end joining (NHEJ) in mammalian cells, which hindered the application of precise genome editing. Recently, a non-viral and PD-1-specific targeted CAR-T cells were generated by CRISPR-Cas9, which showed superior efficacy and safety to eradicate tumor cells in treatment of relapsed/refractory aggressive B cell non-Hodgkin lymphoma [25]. Single-cell analysis exhibited that this manufacturing process produced a higher proportion of memory T cells and enhanced the antitumor immune function of CAR-T cells, which have great potential in competing with or replacing the current virus-based

Table 1 Clinical studies using gene-editing CAR-T cells

ClinicalTrials.gov Identifiers	Targets	Conditions	Gene editing tools	Edited genes	Sponsors and collaborators
NCT04213469	CD19	Relapse/refractory B-Cell Non-Hodgkin Lymphoma (B-NHL)	CRISPR	PD-1	Bioray Laboratories First Affiliated Hospital of Zhejiang University
NCT05381181	CD19	Acute Lymphoblastic Leukemia (ALL), B-NHL	CRISPR	TCR, HLA-I	Bioray Laboratories First Affiliated Hospital of Zhengzhou University
NCT04037566	CD19	ALL, B-Cell, CD19 Positive	CRISPR	HPK1	Xi'An Yufan Biotechnology Co.,Ltd Xijing Hospital
NCT05859997	CD19	Relapse or refractory autoimmune diseases	CRISPR	TCR, HLA-I	Bioray Laboratories Shanghai Changzheng Hospital
NCT03747965	MSLN	Solid Tumor	CRISPR	PD-1	Chinese PLA General Hospital
NCT04976218	EGFR	Solid Tumor	CRISPR	TGF-β receptor II	Chinese PLA General Hospital
NCT05714345	CD19	Relapsed/Refractory Large B Cell Lymphoma	TALEN	TCR, CD52	Allogene Therapeutics
NCT04960579	BCMA	Multiple Myeloma (MM)	Cas-CLOVER™	TCR, β-2 microglobulin	Poseida Therapeutics, Inc. Roche-Genentech
NCT04637763	CD19	Refractory B-NHL	CRISPR	TCR, PD-1	Caribou Biosciences, Inc.
NCT05643742	CD19	B-cell Malignancy	CRISPR	TCR, β-2 microglobulin	CRISPR Therapeutics AG
NCT04142619	CS1	Relapsed/Refractory MM	TALEN	CS1, TCR	Collectis S.A.
NCT04154709	CD19	ALL	CRISPR	TCR, CD52	Nanjing Bioheng Biotech Co., Ltd. Xuzhou Medical University
NCT04538599	CD7	CD7 ⁺ Hematologic Malignancies	CRISPR	CD7, TCR, MHC II	First Affiliated Hospital of Zhejiang University
NCT05377827	CD7	CD7 ⁺ Hematologic Malignancies	CRISPR	CD7, TCR	Wugen, Inc. Washington University School of Medicine

manufacturing process. Meanwhile, through a site-specific mutation to Cas12a, the efficiency of HDR-mediated gene integration and the CD19 CAR knock-in efficiency were improved significantly, suggesting the great potential of Cas12a in T cell engineering [26]. Manufacturing costs and complexity should be drastically reduced to benefit all patients, and nonviral approaches appear to have a unique perspective on clinical application conditions and current good manufacturing practice.

Limitation and safety concerns on gene-editing CAR-T cells. Like most other applications of gene-editing technology, the off-target effects are also the biggest concerns in gene-editing-based CAR-T cells which have potential risk to interfere tumor-suppressor genes or oncogenes. Meanwhile, multiple gene editing such as manufacturing for allogeneic CAR-T cells may cause chromosomal abnormality as well, which has been found in a patient who had received Allogene Therapeutics' TALEN-edited allogeneic CAR-T cells. And this chromosomal aberration is not random but specific to chromosome 14 which is the location of target gene TRAC, suggesting the close correlation with gene editing. Theoretically, the gene editing induced chromosomal abnormality not limited to TALEN technology, each double-strand breaks (DSBs) to the target DNA can generate chromosomal damage, including large-scale deletions, rearrangements and chromothripsis [27]. Thus, the advantage of base and prime editing can be exploited in CAR-T therapy to avoid potential genotoxicities associated with DSBs. Meanwhile, the upcoming iPSCs technology makes it possible to develop a unique batch from a single clone which seems much safer than gene-edited heterogeneous cell pool. Nonetheless, the gene editing-associated genotoxic safety issues should be continuously concerned in clinical application of CAR-T therapy.

Emerging genome-editing applications enable the creation of more-effective CAR-T cells that are cost-effective, safe, durable and available. However, the off-target effects and efficiency are still hurdles along the way for clinical application. Fortunately, improved CRISPR-Cas systems, careful sgRNA designing, and upgraded gene editing tools, such as adenine or cytosine base editors, truncated g-RNAs, ribonucleoprotein delivery, and prime editing, can minimize the off-target effects of the gene editing systems. And the safety and efficacy of more and more gene-editing CAR-T cells have been verified in clinical trials (Table 1). We anticipate that in the coming years, gene editing technologies will expand the versatility of CAR-T cells to be more effective and safer, thus becoming an indispensable living drugs to fulfill more unmet clinical needs.

Data availability

All data generated during this study are included in this published article.

Acknowledgements

We thank Mr. Boxu Lin and Ms. Fangfei Yu for information collection and drawing assistance.

Funding

This work was supported by the National Key R&D Program of China (2018YFA0507001 and 2019YFA0802800).

Author contributions

B.D., J.Z. and M.L. conceived and wrote the manuscript. All authors read and approved the submission of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

- 1 Katti A, Diaz BJ, Caragine CM, *et al.* CRISPR in cancer biology and therapy. *Nat Rev Cancer* 2022; **22**: 259–279.
- 2 Li G, Li X, Zhuang S, *et al.* Gene editing and its applications in biomedicine. *Sci China Life Sci* 2022; **65**: 660–700.
- 3 Neelapu SS, Locke FL, Bartlett NL, *et al.* Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-Cell lymphoma. *N Engl J Med* 2017; **377**: 2531–2544.
- 4 Delhove JM, Qasim W. Genome-edited T cell therapies. *Curr Stem Cell Rep* 2017; **3**: 124–136.
- 5 Carnevale J, Shifrut E, Kale N, *et al.* RASA2 ablation in T cells boosts antigen sensitivity and long-term function. *Nature* 2022; **609**: 174–182.
- 6 Chen Z, Arai E, Khan O, *et al.* *In vivo* CD8⁺ T cell CRISPR screening reveals control by Fli1 in infection and cancer. *Cell* 2021; **184**: 1262–1280.e22.
- 7 Kumar S, Zeng Z, Bagati A, *et al.* CARM1 inhibition enables immunotherapy of resistant tumors by dual action on tumor cells and T cells. *Cancer Discov* 2021; **11**: 2050–2071.
- 8 Ye L, Park JJ, Peng L, *et al.* A genome-scale gain-of-function CRISPR screen in CD8 T cells identifies proline metabolism as a means to enhance CAR-T therapy. *Cell Metab* 2022; **34**: 595–614.e14.
- 9 Legut M, Gajic Z, Guarino M, *et al.* A genome-scale screen for synthetic drivers of T cell proliferation. *Nature* 2022; **603**: 728–735.
- 10 Cherkassky L, Morello A, Villena-Vargas J, *et al.* Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest* 2016; **126**: 3130–3144.
- 11 Stadtmauer EA, Fraietta JA, Davis MM, *et al.* CRISPR-engineered T cells in patients with refractory cancer. *Science*

- 2020; **367**.
- 12 Wang Z, Li N, Feng K, *et al.* Phase I study of CAR-T cells with PD-1 and TCR disruption in mesothelin-positive solid tumors. *Cell Mol Immunol* 2021; **18**: 2188–2198.
 - 13 Si J, Shi X, Sun S, *et al.* Hematopoietic progenitor kinase1 (HPK1) mediates T cell dysfunction and is a druggable target for T cell-based immunotherapies. *Cancer Cell* 2020; **38**: 551–566.e11.
 - 14 Wiede F, Lu KH, Du X, *et al.* PTP1B is an intracellular checkpoint that limits T-cell and CAR T-cell antitumor immunity. *Cancer Discov* 2022; **12**: 752–773.
 - 15 Zhao B, Gong W, Ma A, *et al.* SUSD2 suppresses CD8⁺ T cell antitumor immunity by targeting IL-2 receptor signaling. *Nat Immunol* 2022; **23**: 1588–1599.
 - 16 Yi Y, Chai X, Zheng L, *et al.* CRISPR-edited CART with GM-CSF knockout and auto secretion of IL6 and IL1 blockers in patients with hematologic malignancy. *Cell Discov* 2021; **7**: 27.
 - 17 Eyquem J, Mansilla-Soto J, Giavridis T, *et al.* Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature* 2017; **543**: 113–117.
 - 18 Sachdeva M, Busser BW, Temburni S, *et al.* Repurposing endogenous immune pathways to tailor and control chimeric antigen receptor T cell functionality. *Nat Commun* 2019; **10**: 5100.
 - 19 Levine BL, Miskin J, Wonnacott K, *et al.* Global manufacturing of CAR T cell therapy. *Mol Ther-Methods Clin Dev* 2017; **4**: 92–101.
 - 20 Benjamin R, Graham C, Yallop D, *et al.* Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. *Lancet* 2020; **396**: 1885–1894.
 - 21 Hu Y, Zhou Y, Zhang M, *et al.* CRISPR/Cas9-engineered universal CD19/CD22 dual-targeted CAR-T cell therapy for relapsed/refractory B-cell acute lymphoblastic leukemia. *Clin Cancer Res* 2021; **27**: 2764–2772.
 - 22 Jo S, Das S, Williams A, *et al.* Endowing universal CAR T-cell with immune-evasive properties using TALEN-gene editing. *Nat Commun* 2022; **13**: 3453.
 - 23 Hu Y, Zhou Y, Zhang M, *et al.* Genetically modified CD7-targeting allogeneic CAR-T cell therapy with enhanced efficacy for relapsed/refractory CD7-positive hematological malignancies: a phase I clinical study. *Cell Res* 2022; **32**: 995–1007.
 - 24 Jin G, Chang Y, Harris JD, *et al.* Adoptive immunotherapy: A human pluripotent stem cell perspective. *Cells Tissues Organs* 2023; 1–29, <https://doi.org/10.1159/000528838>.
 - 25 Zhang J, Hu Y, Yang J, *et al.* Non-viral, specifically targeted CAR-T cells achieve high safety and efficacy in B-NHL. *Nature* 2022; **609**: 369–374.
 - 26 Ling X, Chang L, Chen H, *et al.* Improving the efficiency of CRISPR-Cas12a-based genome editing with site-specific covalent Cas12a-crRNA conjugates. *Mol Cell* 2021; **81**: 4747–4756.e7.
 - 27 Sheridan C. Off-the-shelf, gene-edited CAR-T cells forge ahead, despite safety scare. *Nat Biotechnol* 2022; **40**: 5–8.