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Progress and Future Prospects of CRISPR Gene Editing Technology in CAR-T Cell Immunotherapy

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Abstract

Since its inception, chimeric antigen receptor T-cell (CAR-T) therapy has achieved remarkable efficacy in relapsed and refractory hematologic malignancies. However, issues such as low response rates in solid tumors, lengthy and costly autologous product preparation, and insufficient long-term safety data have consistently limited its broader clinical application. The maturation of CRISPR/Cas9 gene editing technology has provided new tools to address these bottlenecks—by simultaneously modifying multiple targets in the T-cell genome, researchers can construct off-the-shelf allogeneic CAR-T products, knock out immune checkpoints to enhance antitumor activity, integrate safety switches to control toxicity, and even expand indications to autoimmune diseases. This article focuses on the technical approaches for optimizing CAR-T cells through gene editing. It reviews the current state of research on three strategies—the construction of universal products, enhancement of therapeutic efficacy, and improvement of safety—and, drawing on the latest clinical data from the fields of hematologic malignancies, solid tumors, and autoimmune diseases, discusses unresolved issues such as off-target effects, T-cell exhaustion, and manufacturing processes. The article also provides an outlook on cutting-edge directions, including in vivo CAR-T cells and iPSC-derived cells.

Keywords

CRISPR/Cas9, gene editing, CAR-T cells, immunotherapy, universal CAR-T

1. Introduction

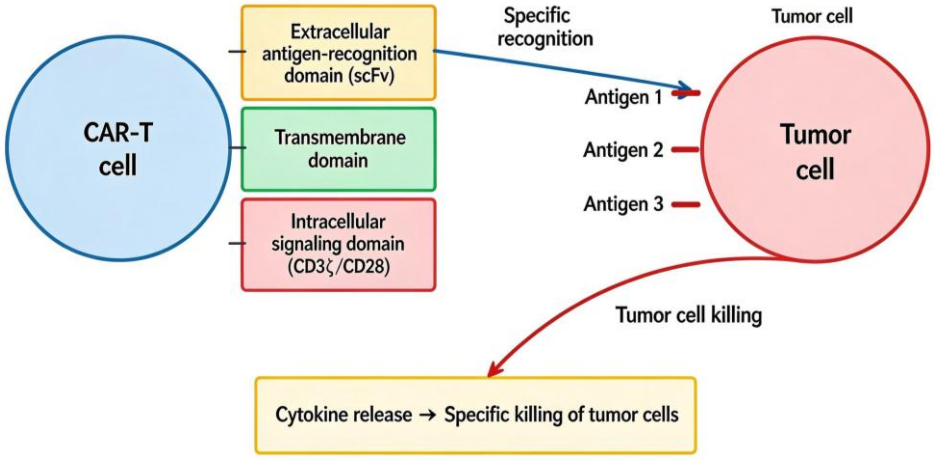
Over the past decade, cancer immunotherapy has fundamentally transformed the treatment landscape for various malignant tumors, with CAR-T cell therapy being the most representative example. Through genetic engineering, chimeric receptors that recognize tumor antigens are expressed on the surface of T cells; CAR-T cells can thus bypass the restrictions of the major histocompatibility complex (MHC) to directly recognize and kill tumor cells [1]. Since the approval of the first CD19-targeted CAR-T product in 2017, this class of therapy has repeatedly demonstrated its efficacy in B-cell malignancies—with initial response rates generally exceeding 80 percent, and many patients who previously had no treatment options achieving long-term disease-free survival [2].

However, despite this success, the shortcomings of CAR-T therapy are equally evident. Currently, all marketed products are autologous; the process from leukapheresis to cell reinfusion typically takes 2 to 4 weeks, and the cost per treatment can reach hundreds of thousands of dollars. Many patients experience disease progression while waiting and thus lose their chance at treatment [3]. Even when preparation is completed,

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some patients have severely compromised T-cell numbers and function following multiple lines of chemotherapy, making it difficult to guarantee the quality of the prepared CAR-T cells. Solid tumors present an even tougher challenge—the tumor microenvironment is densely populated with inhibitory cytokines, immune checkpoint molecules, and physical barriers, making it difficult for infused CAR-T cells to infiltrate and leaving them prone to exhaustion, resulting in response rates that remain consistently low [4]. In addition, the management of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) also requires more refined safety control methods.

Figure 1: Schematic diagram of the structure and mechanism of CAR-T cells



The emergence of CRISPR/Cas9 gene editing technology just responds to these needs. This system is derived from the adaptive immune mechanism of bacteria and archaea. Cas9 nuclease is guided by single guide RNA (sgRNA) to cut DNA at specific sites in the genome to generate double strand breaks (DSBs), and then gene knockout, knock-in or base modification is achieved by means of the cell's own repair pathway [5]. Compared with earlier Zinc-finger nucleases (ZFNs) and TALENs, CRISPR is simple to design, low-cost, and able to edit multiple gene loci at the same time, which makes it rapidly become the most widely used gene editing tool in the field of life sciences [6].

The combination of gene editing and CAR-T can overcome the limitations of existing therapies in many ways. Knockout of T cell receptor (TCR) and CD52 can eliminate the risk of allogeneic T cells causing graft-versus-host disease, while allowing the cells to resist immune clearance in conditioning regimens, thereby achieving off-the-shelf products without donor restrictions [7]. Knockout of immune checkpoint molecules such as PD-1 can enable CAR-T cells to maintain their function for a longer time in the immunosuppressed tumor microenvironment [8]. Genome-wide CRISPR screening can also systematically find new targets that affect the efficacy and durability of CAR-T, providing ideas for the design of next-generation products [9].

In the past two years, a new generation of tools that do not rely on DSBs—such as base editors, prime editors, and epigenetic editors—have gradually matured. The precision and safety of gene editing have reached new heights, creating conditions for more refined modification of CAR-T cells [10]. Meanwhile, several gene-edited CAR-T products have entered the clinical trial phase, yielding encouraging results in hematologic malignancies, solid tumors, and even autoimmune diseases [11]. Against this backdrop, this article systematically reviews research progress on the optimization of CAR-T cells using CRISPR gene editing technology: First, it introduces the technical principles of the CRISPR/Cas9 system and next-generation tools such as base editing, guide editing, and epigenetic editing; subsequently, it elaborates on the core strategies for optimizing CAR-T cells through gene editing from three dimensions: the construction of universal allogeneic CAR-T cells, the enhancement of antitumor efficacy, and the improvement of safety; Next, the current status of applications is analyzed based on the latest clinical data from three major fields: hematologic malignancies, solid tumors, and autoimmune diseases; the paper then discusses unresolved challenges such as off-target effects, T-cell exhaustion, and manufacturing processes; finally, it offers an outlook on cutting-edge directions such as in vivo CAR-T cells and induced pluripotent stem cell (iPSC)-derived cells. This paper aims to provide

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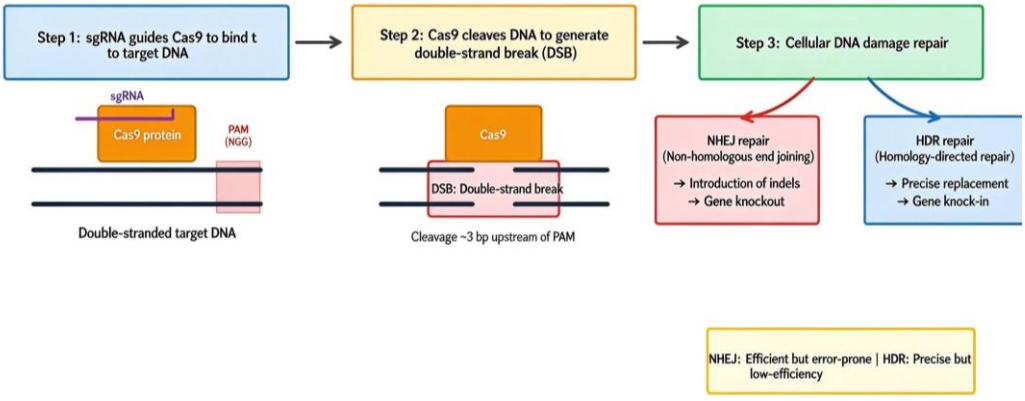
a systematic reference framework for researchers in the field of gene-edited immunotherapy and to advance CAR-T therapy toward greater safety, efficacy, and accessibility.

2. Overview of CRISPR Gene-Editing Technology

2.1 Principles and Development of the CRISPR/Cas9 System

The CRISPR/Cas system is essentially a form of "immune memory" developed by bacteria and archaea over the course of their long evolutionary history—when a bacteriophage invades, the bacterium integrates fragments of the exogenous DNA into the CRISPR array within its own genome; the next time it encounters the same invader, the transcribed crRNA guides the Cas protein to precisely cleave the exogenous DNA. In 2012, the teams led by Doudna and Charpentier simplified this system into a dual-RNA-guided in vitro gene-editing tool, demonstrating that the Cas9 protein from Streptococcus pyogenes could be programmed to cleave any DNA sequence [12]. The following year, the teams led by Zhang Feng and Church reported, almost simultaneously, the successful application of CRISPR/Cas9 in mammalian cells, thereby opening the door to gene editing in eukaryotes [13].

Figure 2: Schematic diagram of CRISPR/Cas9 gene editing technology principle



There are only two core components in this system: Cas9 nuclease and sgRNA. The sgRNA is formed by the fusion of crRNA and tracrRNA, with about 20 nucleotides at the 5' end of the sgRNA locking the target sequence through base complementary pairing, and the stem-loop structure at the 3' end is responsible for binding the Cas9 protein. The recognition of the target sequence also requires the presence of a PAM sequence downstream -- the most commonly used SpCas9 recognizes 5'-NGG-3', and this short sequence is the key marker for Cas9 to distinguish "self" from "non-self" [14]. Upon binding of the sgRNA-Cas9 complex to the correct target site, the HNH domain cleaves the complementary strand and the RuvC domain cleaves the non-complementary strand, leaving a flat-ended DSB about 3 base pairs upstream of the PAM. After the rupture occurs, cells mainly repair the broken ends through two pathways: non-homologous end joining (NHEJ) directly joins the broken ends, which is easy to introduce small insertions or deletions, so it is often used for gene knockout. Homologous recombination repair (HDR) requires exogenous homologous templates to achieve precise gene knock-in or base substitution [15].

2.2 Next-generation Gene Editing Tools

Although CRISPR/Cas9 is useful, the safety risk caused by DSBs is always a sword hanging over the head. During multiple edits, simultaneous breaks at multiple sites can lead to chromosomal translocations, large-scale deletions, or even activation of the p53 pathway, placing edited cells at a selective disadvantage [16]. To circumvent DSBs, researchers have developed a series of milder editing tools.

Base editors were among the first to reach maturity. In 2016, David Liu's team first reported the cytosine base editor (CBE), which fuses nCas9 (a Cas9 nickase that cuts only one DNA strand) with a cytosine deaminase; this allows for the conversion of C-G to T-A without generating DSBs; Subsequently, they developed the adenine base editor (ABE), which enables the conversion of A-T to G-C [17]. These tools feature high editing efficiency and minimal byproducts, and have already demonstrated significant advantages in

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therapeutic T-cell engineering—a 2025 study in PNAS directly compared quadruple-edited allogeneic CAR-T cells generated using ABE and conventional Cas9, finding that ABE-edited cells exhibited stronger proliferative capacity under in vitro antigen stress and showed significantly better tumor control and survival times in a mouse leukemia model compared to the Cas9-edited group, precisely because the genotoxic damage caused by Cas9-induced DSBs impairs cellular adaptability [18].

The prime editor, also developed by Liu's team, fuses nCas9 with a reverse transcriptase; guided by pegRNA, it directly writes a reverse transcription template containing the target modification into the target site. Theoretically, the prime editor can achieve all 12 types of base substitutions as well as small-fragment insertions and deletions, offering a broader editing range than base editors and, similarly, does not rely on DSBs [19]. In 2026, the KNIT Editing technology reported in *Nature* by Wang Haifeng's team at Tsinghua University went a step further, achieving precise DNA insertion on a scale of thousands of bases through targeted cleavage by nickases, providing a new tool for the site-specific integration of CAR genes [20].

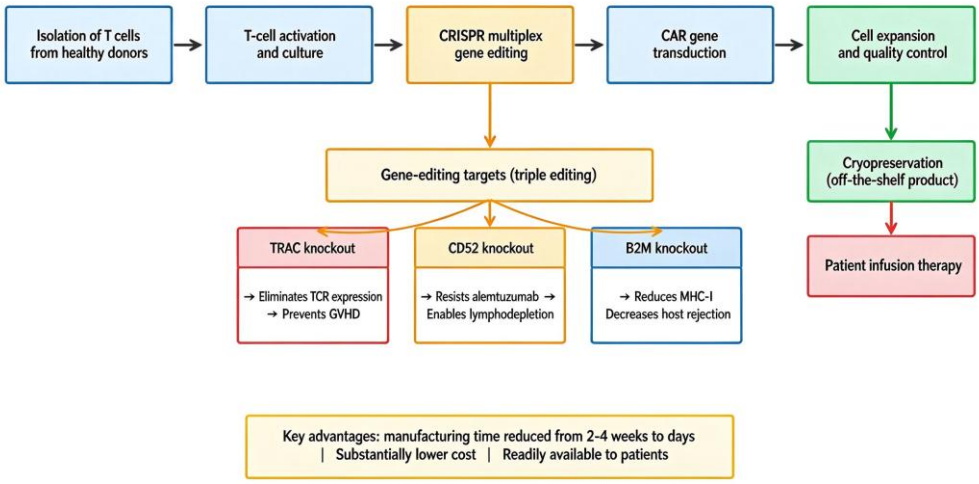
Epigenetic editors go the other way - instead of changing the DNA sequence itself, they carry DNA methyltransferases or histone modifying enzymes through dCas9 to regulate the expression state of genes at specific sites. This regulation is reversible and theoretically safer than permanent gene knock-out. The CRISPRoff/CRISPRon platform reported by Nature Biotechnology in 2026 achieved long-term silencing or activation of multi-locus genes in primary human T cells, and the effect of tumor control in vivo was significantly improved, without the introduction of DSBs in the whole process [21].

3. Strategies for Optimizing CAR-T Cells Through CRISPR Gene Editing

3.1 Construction of Universal CAR-T

The idea of universal CAR-T (UCAR-T) is straightforward: instead of the patient's own T cells, cells are collected from healthy donors, prepared into allogeneic products after gene editing to eliminate genes related to immune rejection, frozen in advance, and available at any time when the patient needs it. This not only reduces the preparation cycle from weeks to days, but also significantly reduces costs through large-scale production [22].

Figure 3: Flowchart of the Strategy for Constructing Universal Allogeneic CAR-T Cells



For allogeneic T cells to survive safely and function effectively within a patient's body, two immunological hurdles must be overcome. The first is graft-versus-host disease (GVHD)—the endogenous TCR on the surface of donor T cells recognizes the patient's normal tissues as "foreign" and attacks them, causing symptoms ranging from mild rashes and diarrhea to severe multi-organ failure. The solution is to knock out the T-cell receptor alpha-chain constant region (TRAC) gene, preventing the TCR from assembling on the cell surface. TRAC is a single-copy gene with low homology to other regions of the genome, making it an ideal target for gene editing [23].

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The second challenge is host-versus-graft reaction (HVGR)—residual T cells and NK cells in the patient's body recognize and eliminate allogeneic T cells, resulting in a short survival time for the reinfused CAR-T cells and reduced therapeutic efficacy. There are two primary strategies to address this: knocking out CD52 makes donor cells resistant to killing by the anti-CD52 monoclonal antibody (alemtuzumab), allowing alencomab to be used during pretreatment to eliminate the patient's T cells and NK cells without harming the donor CAR-T cells [24]; knocking out β2-microglobulin (B2M) eliminates the surface expression of MHC class I molecules, reducing the recognition of allogeneic cells by the patient's T cells. Some products further knock out CIITA to eliminate MHC class II molecules, thereby further delaying rejection reactions [25].

Currently, the CRISPR-edited allogeneic CAR-T cell product making the fastest clinical progress is CRISPR Therapeutics' zugo-cel (formerly CTX112), which targets CD19 and simultaneously knocks out TRAC and CD52. Data released in 2026 showed that in patients with relapsed or refractory large B-cell lymphoma, the overall response rate in the 600-million-cell dose group reached 90%, with a complete response rate of 70%; two-thirds of patients maintained complete remission at 1-year follow-up. The efficacy was comparable to that of autologous products, and no unexpected GVHD events occurred [26]. In China, Bioheng Biotech's CTA101 follows a dual-target approach targeting CD19 and CD22, also with TRAC and CD52 knocked out; in patients with relapsed or refractory acute lymphoblastic leukemia, the complete remission rate reached 83.3% 28 days after infusion [27].

3.2 Enhancing the Antitumor Efficacy of CAR-T Cells

The tumor microenvironment suppresses CAR-T cells in multiple ways: immune checkpoint molecules are persistently upregulated, inhibitory cytokines pervade the environment, and regulatory T cells and tumor-associated macrophages surround the CAR-T cells in multiple layers. As a result, infused T cells are easily suppressed, experience accelerated exhaustion, and cannot survive for long in the body [28]. Gene editing offers various approaches to overcome these suppressive mechanisms.

Immune checkpoint knockout is the most direct approach. PD-1 is one of the most important inhibitory receptors on the surface of T cells; when it binds to PD-L1 on the surface of tumor cells, it transmits inhibitory signals that suppress T-cell proliferation and cytokine secretion. Knocking out the PD-1 gene in CAR-T cells can lift this layer of inhibition at the tumor site. In 2020, two Phase I clinical trials demonstrated for the first time the safety and feasibility of PD-1-knockout CAR-T therapy in cancer patients [29]. In addition to PD-1, novel checkpoints such as TIGIT, LAG-3, and TIM-3 are also being tested; the combined knockout of multiple checkpoints may produce synergistic effects [30].

Whole-genome CRISPR screening offers an "unbiased" approach to target discovery. In a study published in *Nature* in August 2026, a team from the Gladstone Institute and UCSF established the world's first in vivo genome-wide CRISPR screening platform to systematically identify genes regulating CAR-T cell infiltration and function in solid tumor models. The screening results pointed to two pathways that had not previously received sufficient attention: the P2RY8-Gα13 pathway mediates the inhibition of T-cell infiltration by tumor-secreted ligands, while GNAS (Gαs) integrates multiple inhibitory signals to drive T-cell exhaustion. Knocking out GNAS alone enhances CAR/TCR-T cell-mediated killing of solid tumors in multiple models, while the synergistic effect of dual knockout of P2RY8 and GNAS is even more pronounced—in many mouse models where standard CAR-T cells are ineffective, cells with the dual knockout successfully cleared tumors [31]. Such screening efforts provide concrete candidate targets for the design of next-generation CAR-T therapies for solid tumors.

In addition, editing genes related to T-cell differentiation can help maintain the memory T-cell phenotype, allowing the cells to survive longer in vivo; knocking out exhaustion-related transcription factors such as TOX and NR4A1 has been shown to delay the T-cell exhaustion process [32]. These strategies each have their own focus and are likely to appear in next-generation products in the form of combined edits.

3.3 Improving the Safety of CAR-T Cells

The management of toxicity associated with CAR-T therapy has always been a key focus in clinical practice. Severe cases of CRS and ICANS can be life-threatening, while "on-target off-tumor" toxicity—caused by CAR-T cells recognizing target antigens on normal tissues—is equally challenging. Gene editing offers several viable approaches to enhance safety.

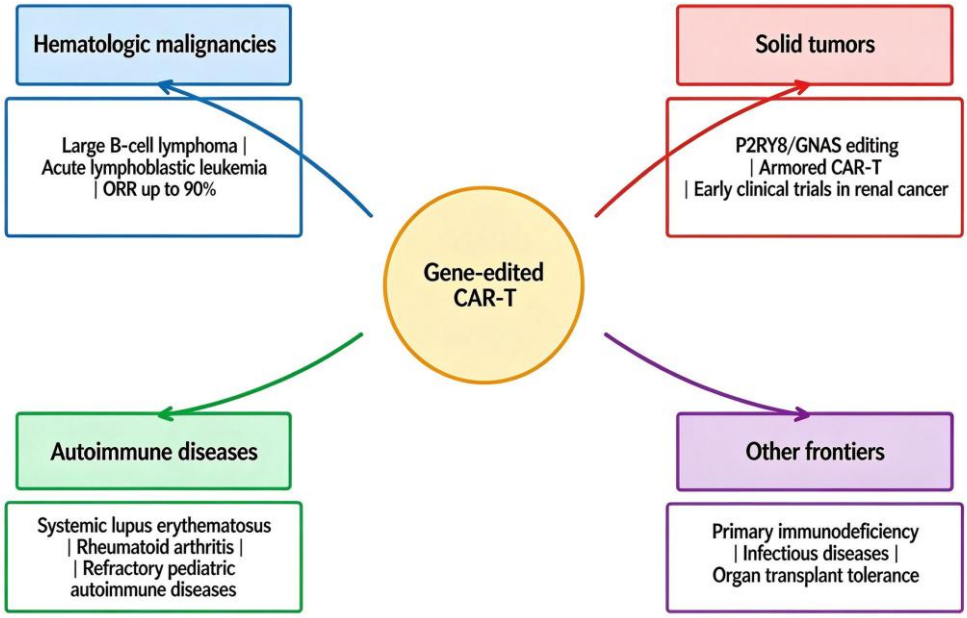
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Safety switches (suicide genes) are the most commonly used approach. By integrating the inducible caspase-9 (iC9) gene into the CAR-T cell genome, a small-molecule inducer can be administered in the event of severe toxicity to activate the caspase-9 pathway, inducing apoptosis in CAR-T cells and thereby rapidly controlling the toxicity [33]. Another approach involves expressing truncated CD19 or EGFR (EGFRt) on the cell surface as a marker; when necessary, CAR-T cells can be eliminated via antibody-dependent cellular cytotoxicity using the corresponding antibody.

Antigen-specific optimization reduces the risk of toxicity at the source. Dual-target CAR design can not only reduce tumor antigen escape but also employ "AND-gate" logic, requiring the simultaneous recognition of two antigens to activate T cells, thereby avoiding collateral damage to normal tissues that express only a single antigen. More complex logic-gated CARs also include "OR-gates" and "NOT-gates," which enable fine-tuned regulation of T-cell activation through synthetic biology gene circuits [34].

4. Progress in Clinical Application Research

Figure 4: Overview of Clinical Applications of Gene-Edited CAR-T Cells



4.1 Hematologic Malignancies

Hematologic malignancies represent the most mature field for clinical research on gene-edited CAR-T cells. Among B-cell malignancies, allogeneic CAR-T cells targeting CD19 have demonstrated results comparable to those of autologous products—as mentioned earlier, Zugo-Cel achieved an ORR of 90% and a CRR of 70% in large B-cell lymphoma, with manageable safety profiles [26]. In addition to large B-cell lymphoma, allogeneic CAR-T cells targeting CD19 have also shown promising results in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL); CTA101 achieved a CR rate of 83.3% in relapsed/refractory (r/r) ALL [27].

T-cell malignancies are an area where the unique advantages of allogeneic CAR-T therapy are particularly evident. When preparing CAR-T cells targeting CD7 or CD5 using autologous T cells, a problematic issue arises: the CAR-T cells themselves express these target antigens, leading to fratricide—where cells kill one another—which adversely affects both preparation efficiency and product quality. Allogeneic CAR-T cells, however, can have the target antigen gene knocked out via gene editing, fundamentally preventing fratricide while simultaneously knocking out the TCR to prevent GVHD. At present, the safety and efficacy of allogeneic CAR-T targeting CD7 and CD5 have been initially verified in T-cell acute lymphoblastic leukemia and T-cell lymphoma [35].

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Research on myeloid neoplasms is also advancing. Allogeneic CAR-T targeting CD123 has shown initial anti-leukemic activity in acute myeloid leukemia (AML). A 2025 study reported that base-edited universal donor CAR-T targeting CD33 and CLL-1 produced significant antitumor effects in AML patient–derived xenograft models and achieved CD7 CAR-T compatibility by removing the common CD7 antigen. It provides an idea for "mixing and matching" different CAR-T products according to the antigen profile of patients [36].

4.2 Solid Tumors

Solid tumors are the greatest challenge for CAR-T therapy, and it is precisely where gene editing can be most effective. Solid tumor microenvironment has strong immunosuppression, dense physical barrier and high antigen heterogeneity, which makes it difficult for standard CAR-T cells to effectively infiltrate and continue to function [37].

Gene editing enhances CAR-T against solid tumors from multiple perspectives. The aforementioned P2RY8 and GNAS double knockout is a typical example, and the edited cells have significantly enhanced infiltration and killing ability in a variety of solid tumor models [31]. The "armored CAR-T" strategy enables CAR-T cells to secrete pro-inflammatory cytokines such as IL-12 and IL-18 on their own, or to express a dominant-negative TGF-β receptor II to counteract inhibitory signals in the microenvironment [38]. Other studies have enabled CAR-T cells to secrete anti-PD-1 single-chain antibodies, blocking the PD-1/PD-L1 axis locally within the tumor. This not only restores the CAR-T cells' own function but also activates endogenous T-cell responses, yielding better results than systemic combination therapy [39].

In terms of clinical translation, gene-edited allogeneic CAR-T cells have begun to be applied to solid tumors. An allogeneic CAR-T product targeting CD70, ALLO-316, showed preliminary antitumor activity in patients with advanced clear cell renal cell carcinoma in a phase I study reported in 2026. This product uses TALEN editing to knock out TCR and CD52, while simultaneously being designed to target and eliminate host T cells expressing CD70—cells that could potentially mediate allogeneic rejection, effectively enabling the product to "protect itself" [40]. Although gene-edited CAR-T therapy for solid tumors is still in the early clinical stages overall, continuous breakthroughs in preclinical research have laid the foundation for future translation.

4.3 Autoimmune Diseases

In recent years, the indications for CAR-T therapy have been expanding from cancer to autoimmune diseases, which may be one of the most exciting directions in this field. Anti-CD19 CAR-T cells have demonstrated significant efficacy in various refractory autoimmune diseases by eliminating B cells that produce autoantibodies. Gene-edited allogeneic CAR-T has a natural advantage in this field: patients with autoimmune diseases often receive long-term immunosuppressive therapy, and the quality of autologous T cells is a concern, while allogeneic products can provide cells with more stable quality [41].

In August 2026, Huji Xu team from the Naval Medical University and Xin Lin team from Tsinghua University published a blockbuster study in Nature Medicine, which reported the results of YTS109, an allogeneic universal CD19 STAR-T cell product, in the treatment of refractory systemic lupus erythematosus with lupus nephritis for the first time in the world. After treatment, clinical symptoms were significantly improved, urinary protein was decreased, and no severe GVHD events occurred. The feasibility and safety of allogeneic universal CAR-T in the treatment of autoimmune diseases were verified [42].

Good news has also emerged in the field of rheumatoid arthritis (RA). A 2026 study in *Nature Medicine* reported the world's first clinical trial of CAR-T therapy for RA: all six patients with refractory RA experienced a significant reduction in disease activity following anti-CD19 CAR-T treatment, and by the end of the observation period, three patients had completely discontinued RA medications, providing preliminary validation of CAR-T therapy's potential to achieve "drug-free remission" in autoimmune diseases [43]. Progress has also been made in pediatric refractory autoimmune diseases -- a study in 2026 reported that anti-CD19 CAR-T therapy for a variety of pediatric refractory autoimmune diseases, including systemic lupus erythematosus, juvenile idiopathic arthritis, and autoimmune encephalitis, significantly improved clinical symptoms during B cell depletion with controllable safety [44].

In addition to CAR-T, gene editing technology has also been used to engineer regulatory T cells (Tregs). In a study published in *Nature* in 2026, RBPJ, a key negative regulator of FOXP3, was identified through

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genome-wide CRISPR screening. Knockout of RBPJ could significantly enhance the stability and immunosuppressive ability of inducible Treg, and the efficacy was equivalent to that of natural thymus-derived Treg in the GVHD model induced by human PBMC [45]. In the same year, research published in *Cell Stem Cell* developed an in vivo Tfh Cell reprogramming strategy based on lipid nanoparticles, which transformed follicular helper T cells into regulatory T cells expressing Foxp3 in vivo, and restored immune tolerance in a mouse model of autoimmune hepatitis [46].

5. Cutting-Edge Technology Directions

5.1 In Vivo CAR-T

The process of preparing CAR-T cells in vitro is complex, costly, and time-consuming, which is a key bottleneck limiting access to treatment. The concept of in vivo CAR-T was developed precisely to circumvent this bottleneck—rather than extracting cells for in vitro modification, gene-editing tools and CAR templates are delivered directly into the patient's body, allowing T cells to be reprogrammed into CAR-T cells in situ [47].

In April 2026, a collaborative team involving UCSF reported a breakthrough method in *Nature* known as the "in vivo pharmaceutical factory." They designed a dual-vector delivery system: using an enveloped delivery vehicle (EDV) to deliver the CRISPR-Cas9 ribonucleoprotein (RNP), while simultaneously using an adeno-associated virus (AAV) to deliver the CAR template, thereby efficiently and specifically integrating the CAR gene into the TRAC locus of T cells. In B cell hypoplasia, hematological tumors and solid tumor models of humanized mice, this system has generated therapeutic levels of CAR-T cells in vivo with remarkable efficacy [48]. This work marks a key step toward making cell and gene therapies more accessible and convenient.

Of course, in vivo CAR-T cells are still some distance from clinical application. Questions regarding the targeting specificity of the delivery system, gene-editing efficiency, immunogenicity, and long-term safety all need to be addressed. However, with the continuous optimization of delivery technology and editing tools, in vivo CAR-T has entered the early clinical validation stage, which is expected to further mature and fundamentally change the accessibility of cell therapy in the future.

5.2 iPSC-derived CAR-T

iPSC technology provides an alternative path for standardized large-scale production of CAR-T cells. iPSC have unlimited proliferation ability, can be expanded in vitro for a long time and maintain genome stability, and the threshold for gene editing is low, which is suitable for complex multi-gene modification. CAR-T cells differentiated from gene-edited iPSCs have uniform phenotype and function, which are suitable for standardized large-scale production, and the cost is expected to be further reduced [50].

Much progress has been made in the research of iPSC-derived CAR-T. In the United States, iPSC-derived CAR-T therapy has entered clinical studies in the field of autoimmune diseases and has obtained preliminary data. In Europe, GMP grade iPSC cell banks have been established, laying the foundation for standardized production. Domestic clinical research results of iPSC-derived cardiomyocytes in the treatment of heart failure were published in Nature, which also proved the clinical feasibility of iPSC-derived cell therapy [51].

Challenges remain. The efficiency and maturity of iPSC differentiation into functional T cells are not yet optimal; the oncogenic risk of differentiated cells must be strictly controlled; and the persistence and functional efficacy of iPSC-derived T cells in vivo still need to be validated. However, with the optimization of differentiation protocols and the refinement of safety strategies, iPSC-derived CAR-T cells are likely to become a key direction for next-generation universal cell therapy [52].

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6. Challenges and Limitations

6.1 Safety Issues of Gene Editing

Off-target effect is the most concerned safety hazard of CRISPR gene editing. When there is a local base mismatch between the sgRNA and the non-target sequence, Cas9 may still be activated and cleave DNA, resulting in unplanned gene editing. Although the overall specificity of CRISPR/Cas9 is high, there may still be off-target sites in the whole genome, and the risk is further superimposed when multiple edits are made [53]. If the off-target mutation happens to fall on the proto-oncogene or tumor suppressor gene, it may lead to malignant transformation of cells, with disastrous consequences.

There are many methods to reduce the risk of off-target, such as high-fidelity Cas9 variants (SpCas9-HF1, eSpCas9, HypaCas9, etc.), optimized sgRNA design algorithms, and shortening the time of Cas9 in cells with ribonucleoprotein delivery, which can reduce the off-target risk to a certain extent. The new generation of base editors and guide editors are independent of DSBs, and the risk of chromosomal translocation and large fragment deletion is significantly reduced, but the deaminase domain may still cause off-target editing at the RNA level, which needs further optimization [54]. In addition, the detection methods and standards for off-target effects have not been completely unified, and the detection platforms and thresholds used in different studies are quite different, which makes it difficult to compare findings across studies. Therefore, it is urgent to establish standardized off-target assessment guidelines [55].

In addition to off-target, the genotoxicity caused by DSB itself cannot be ignored. Cas9-induced DSBs activate the p53 DNA damage repair pathway, which puts the edited cells at a selective disadvantage and may even induce chromosomal translocations and large deletions. The aforementioned 2025 PNAS study clearly pointed out that Cas9-induced genotoxicity would impair the fitness of gene-edited human CAR-T cells, thereby reducing therapeutic efficacy, beyond the known risks of chromosomal translocation and carcinogenesis [18]. This is also an important reason why DSB-free editing techniques are preferred in therapeutic T cell modification.

Immunogenicity is another issue that is easily overlooked. Cas9 protein is derived from bacteria, and there may already be humoral and cellular immune responses against Cas9 in the human body, which may affect the efficiency of gene editing and even cause immune-related adverse reactions. Viral vectors and other reagents used in the in vitro editing process may also pose immunogenicity concerns. How to reduce the immunogenicity of gene editing components is a subject that must be faced in clinical translation [56].

6.2 T Cell Exhaustion and Insufficient Persistence

T cell exhaustion is a dysfunctional state induced by chronic antigen stimulation, which is characterized by decreased proliferation ability, decreased secretion of effector cytokines, and persistent high expression of inhibitory receptors such as PD-1/TIM-3/LAG-3. In CAR-T therapy, especially in the treatment of solid tumors, the continuous stimulation of tumor antigens combined with inhibitory signals in the microenvironment greatly accelerates the exhaustion process of CAR-T cells, which is the core factor restricting the efficacy and durability in vivo [57].

The molecular mechanism of exhaustion involves a complex transcriptional regulatory network. Under chronic stimulation, nuclear factor of activated T cells (NFAT) shifts from forming synergistic heterodimers with AP-1 to forming homodimers, directly promoting the expression of inhibitory receptors, such as PD-1, LAG-3 and TIM-3. This dysfunctional program is further reinforced and maintained by downstream transcription factors such as IRF4, BATF and NR4A1 [58]. As a key transcriptional regulator of exhausted T cells, TOX plays a central role in the establishment and maintenance of the exhaustion program; knocking out TOX can reverse the exhaustion phenotype to some extent.

The issue is that T-cell exhaustion is not merely a simple change in the expression of a few genes, but rather a profound program involving epigenetic remodeling. Simply knocking out a single immune checkpoint or a single exhaustion-related transcription factor often fails to fully reverse the exhaustion phenotype. How to fundamentally prevent or reverse CAR-T cell exhaustion through combined multi-gene editing and epigenetic regulation is a direction that future research needs to address [59].

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6.3 Production Process and Accessibility

The original intention of universal allogeneic CAR-T is to reduce cost and improve accessibility, but its production process is still complex and expensive. The preparation process involves multiple steps such as T cell isolation and activation, gene editing component delivery, CAR gene transduction, cell expansion, and quality testing. The standardized detection and control of key quality attributes such as gene editing efficiency, off-target rate, and cell phenotype uniformity are still facing challenges [60].

The delivery of gene-editing components also has shortcomings. Electroporation is simple but has an effect on cell viability, and the editing efficiency of T cells from different donors varies greatly. Viral vectors (lentivirus, retrovirus) have high transduction efficiency but carry a risk of insertion mutation, and the production cost is not low. AAV vectors are commonly used for HDR-mediated gene knock-in template delivery, but their genome capacity is limited, and high doses of AAV may also trigger immune responses and hepatotoxicity. The development of more efficient, safer and more standardized delivery systems is the key to reducing production costs and improving product quality [61].

There is also an easily overlooked issue: the persistence of universal CAR-T cells in vivo is usually only 1 to 3 months, which is significantly shorter than that of autologous CAR-T cells, to some extent limiting the long-term efficacy. How to optimize and enhance the in vivo persistence of allogeneic CAR-T through gene editing without sacrificing safety is a problem that needs continuous research [62].

7. Summary and Prospect

The combination of CRISPR gene editing and CAR-T cell therapy is reshaping the landscape of tumor immunotherapy and autoimmune disease treatment. Simultaneous multi-gene editing has propelled universal allogeneic CAR-T products from concept to clinical application, achieving efficacy comparable to autologous products in hematologic malignancies and providing a practical pathway to address treatment accessibility issues. Genome-wide CRISPR screening has unearthed new therapeutic enhancement targets such as P2RY8 and GNAS, which brings new possibilities to break through the bottleneck of solid tumors. The maturation of next-generation tools—including base editing, prime editing, and epigenetic editing—has further enhanced the precision and safety of editing, making more refined modification of CAR-T cells a reality. At the clinical application level, the indications for gene-edited CAR-T cells have expanded from hematologic malignancies to solid tumors and autoimmune diseases, demonstrating the potential to achieve “drug-free remission” in refractory conditions such as systemic lupus erythematosus and rheumatoid arthritis.

At the same time, this field is far from reaching a stage where we can rest easy. Off-target effects and DSB-related genotoxicity remain core safety concerns limiting clinical translation, requiring more precise editing tools and a more standardized safety evaluation system. T cell exhaustion and insufficient persistence in vivo limit the long-term efficacy, so it is necessary to further explore the mechanism and develop intervention strategies from the transcriptional regulation and epigenetic level. The complexity and cost of the production process also need to be gradually solved through technological innovation and large-scale production.

Looking ahead, several directions are worth focusing on. DSB-free editing technology is likely to gradually replace the traditional Cas9 nuclease as the mainstream tool for therapeutic T cell editing, and the clinical translation of base editing and epigenetic editing will be accelerated. In vivo CAR-T technology is expected to fundamentally transform the manufacturing model of cell therapy. With the optimization of delivery systems, it will become possible to generate therapeutic CAR-T cells directly within the patient’s body, leading to a qualitative improvement in both treatment costs and accessibility. iPSC-derived CAR-T cells will drive the standardization and large-scale production of cell products; combined with multi-gene editing technologies, they are expected to lead to the development of off-the-shelf, multifunctional next-generation cell therapy products. Artificial intelligence and machine learning will play an increasingly important role in sgRNA design, off-target prediction, and editing result prediction, which will promote the development of gene editing to a more accurate and intelligent direction. In addition, the application scope of gene-edited CAR-T will continue to expand. In addition to tumor and autoimmune diseases, it also has considerable potential in the fields of infectious diseases, organ transplantation tolerance induction, cardiovascular diseases and so on.

In general, CRISPR gene editing is transforming CAR-T cell therapy from an expensive and complex individualized therapy to a safer, more effective, and more accessible standardized treatment. With the

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continuous progress of technology and the in-depth progress of clinical research, gene-edited CAR-T cells are expected to become an important treatment option for a variety of refractory diseases in the next decade.

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Conflicts of Interest

The authors declare no conflict of interest.

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