



REVIEW ARTICLE

CRISPR, CAR-T, and NK: Current applications and future perspectives

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Abstract Chimeric antigen receptor T (CAR-T) cell therapy represents a breakthrough in personalized cancer treatments. In this regard, synthetic receptors comprised of antigen recognition domains, signaling, and stimulatory domains are used to reprogram T-cells to target tumor cells and destroy them. Despite the success of this approach in refractory B-cell malignancies, the optimal potency of CAR T-cell therapy for many other cancers, particularly solid tumors, has not been validated. Natural killer cells are powerful cytotoxic lymphocytes specialized in recognizing and dispensing the tumor cells in coordination with other anti-tumor immunity cells. Based on these studies, many investigations are focused on the accurate designing of CAR T-cells with clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system or other novel gene editing tools that can induce hereditary changes with or without the presence of a double-stranded break into the genome. These methodologies can be specifically focused on negative controllers of T-cells, induce modifications to a particular gene, and produce reproducible, safe, and powerful allogeneic CAR T-cells for on-demand cancer immunotherapy. The improvement of the CRISPR/Cas9 innovation offers an adaptable and proficient gene-editing capability in activating different pathways to help natural killer cells interact with novel CARs to particularly target tumor cells. Novel achievements and future challenges of combining next-generation

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CRISPR-Cas9 gene editing tools to optimize CAR T-cell and natural killer cell treatment for future clinical trials toward the foundation of modern cancer treatments have been assessed in this review.

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Introduction

Patients' immune system usage to target and kill cancer cells has become a promising tool for cancer treatment.¹ T-cell therapy approval may be associated with challenges including the limitation of T-cell isolation, expansion, and transplantation into cancerous patients.² This new therapy is based on the use of peripheral blood T cells to become transgenic T cells and chimeric antigen receptor T (CAR-T) cells (Fig. 1).³ CAR-T cells, known as living drugs, have been studied for several years.⁴ Studies have shown the significant success of CAR-T cell therapy in several solid tumors and hematological malignancies. CAR T-based therapy against hematological malignancies is expected to become more widespread, especially for patients with B-cell acute lymphoblastic leukemia.⁴ Until now, the US Food and Drug Administration (FDA) has approved several CAR-T cell drugs. (i) Idecabtagene vicleucel (Abecma) is an autologous CAR-T cell drug that is programmed for patients with relapsing or refractory myeloma.⁵ (ii) Lisocabtagene maraleucel (Breyanzi) is a CAR-T cell drug specific for patients with relapsing or refractory massive B-cell lymphoma.⁶ (iii) Tecartus is a CAR-T cell for patients with relapsing or refractory mantle cell lymphoma.⁷ (iv) Kymriah is the first CAR-T cell drug that is specific for pediatric and young adult patients with relapsing or refractory B-cell acute lymphoblastic leukemia.⁸ (v) Yescarta is a CAR-T cell drug designed for patients with advanced B-cell lymphoma.⁹ CAR-T cells have several limitations and challenges. Despite the tremendous clinical success of CAR-T cell therapy in hematological malignancies, several obstacles and challenges limit acceptable therapeutic results. CAR-T cells in some patients, especially chronic lymphocytic leukemia, have more limited proliferation and expansion.¹⁰ In addition, the utility of the patient's autologous T cells may prevent the success of CAR-T cell therapy.¹¹

In some types of cancer, time limitation for some patients is a challenge due to the time it takes to ready the CAR-T cell treatment. In addition, it is currently difficult and impractical to collect acceptable numbers of T cells from patients who are under hematological treatments due to chemotherapy-induced lymphopenia or underlying infection.^{12,13} Also, there are other challenges related to CAR-T cell therapy, including tumor invasion, T cell inhibition, antigen rejection, low determination, and genetic mutations.¹⁴ Importantly, barriers such as specific entry and exit criteria set by clinical trials, as well as the development and expansion costs have limited the number of patients receiving this treatment.¹⁵ In addition, CAR-T therapy has been used against malignant tumors and

appears to be a promising approach. So far, the FDA has not approved any CAR-T cell drug for solid tumors, which shows that the challenges in solid tumors are much more critical and require a comprehensive review.¹⁶ With the appearance of genome editing tools, such as transcription activator-like effector nucleases, zinc finger nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) framework, there is an opportunity to address some of the obstacles around CAR-T cell treatment.^{17,18} Genome editing tools can edit genetic materials through gene substitution, gene addition, gene deletion, and gene manipulation.¹⁹ CRISPR has performed better than the other two genome editing tools because (i) it is an easier tool with higher efficiency; (ii) CRISPR/Cas9 recognizes DNA sites through the RNA-DNA interaction; (iii) it provides a simple way to manipulate multiple target DNAs simultaneously (high-throughput manipulation); and (iv) it is a cost-effective innovation.²⁰ After all, an overview of CRISPR/Cas9 innovation, CAR-T cell breakthroughs, natural killer (NK) cell achievements, and their challenges will be provided.

CRISPR gene editing tool

CRISPR-Cas9 technology has provided a simple and precise method for cell, tissue, and whole organism editing with many applications.^{21,22} The origin of this technology is a prokaryotic immunity system against viruses.²³ This system has been tested in several human cells, including primary immune cells such as T cells and NK cells.²⁴ The CRISPR-Cas9 system contains two main domains to make mutations in DNA. An enzyme called Cas9 (this enzyme acts as a pair of molecular scissors that cut dsDNA at a specific location in the genome and creates a double-strand break.²⁵ The other part is a piece of RNA (called a guide RNA or gRNA).²⁶ This piece contains a pre-designed ribonucleic acid sequence that is about 20 bases long.²⁷ This RNA-DNA scaffold leads the Cas9 enzyme to bind in the DNA. Cas 9 removes double-stranded DNA breaks by following the gRNA in the same part of the DNA strand. The DNA cleavage by Cas9 results in a double-stranded DNA break that is repaired by two pathways: non-homologous end-joining which is an error-prone system (NHEJ) pathway and the higher precision homology-directed repair pathway.²⁸ The NHEJ repair pathway is the most active repair mechanism and often causes small nucleotide insertions or deletions (indels) at the double-stranded DNA break site.^{29,30} Since NHEJ-mediated double-stranded DNA break repair is a nonspecific cleavage system, it has important practical implications such as resulting in a pool of different mutations.^{31,32} NHEJ frequently causes

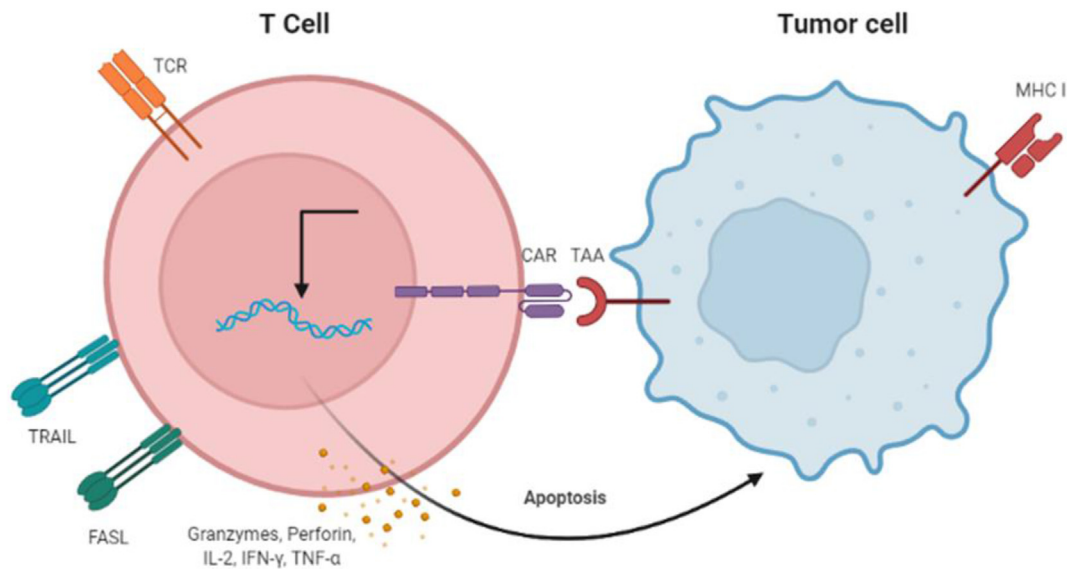


Figure 1 Schematic view of the interaction between T cells and Tumor cells.

small deletion or insertion errors in the target DNA. Homology-directed repair can be used for precise gene editing by inserting a specific DNA template (single-stranded or double-stranded) into the desired site.^{33,34} To fully exploit the editing potential of CRISPR/Cas9, they must be successfully delivered to target cells or tissues using appropriate carriers.^{35,36} Recombinant viral vectors have been developed by exploiting the capacity of viruses to transfer exogenous genetic material into cells to generate beneficial properties in the infected cells/tissues.^{37–39} Safety issues remain a major bottleneck for the widespread clinical use of viral vectors as stable expression systems.⁴⁰ Non-viral vectors have emerged as a promising alternative for cancer therapy owing to their immunogenicity, high biocompatibility, and efficient delivery.³⁵ Nanotechnology-based drug delivery systems have developed broad applications of CRISPR/Cas9 therapy, promoted safety issues, and provided a practical approach to overcome the challenges of viral vectors.^{41,42} On the other hand, T cell exhaustion, CAR T cell inviability,⁴³ and the autologous nature of products have limited the safety, efficiency, and availability of new therapeutic methods.^{16,44–46} Many of these limitations can be overcome using the CRISPR-Cas9-based gene editing tool as an easy and available method.⁴⁷ Accordingly, current research efforts are focused on precise CAR T cell engineering with conventional CRISPR-Cas9 systems or novel editors that can generate the desired mutations with or without inducing a double-stranded DNA break into the genome.⁴⁸ These tools and strategies can be directly applied to target negative regulators of T cell function, insert therapeutic genes to specific loci, and generate universal CAR allogeneic T cell products for on-demand immunotherapy (Fig. 2).⁴⁹ This review evaluates several ongoing and future directions of combining next-generation CRISPR-Cas9 gene editing tools with synthetic biology to enhance CAR T-cell therapy for future clinical trials toward a new cancer treatment paradigm.^{50,51}

CAR-T cell therapy

Cancer therapy has been one of the biggest challenges in medical sciences from the past until now.^{52–54} Due to the treatment complications and different cancer cell reactions with various treatments, it is very important to choose the most appropriate therapy method based on the patient's condition.^{55,56} One of the most effective and promising methods is CAR-T cell therapy.^{57,58} CAR-T cell therapy is a kind of immunotherapy in which, using the ability of immune cells to identify foreign agents, it is possible to kill cancerous cells without harming other normal cells in the body.^{59,60} In general, CAR-T-cell therapy is a method of collecting immune T cells from the blood and modifying them in the laboratory to fight cancer cells and re-injecting them into the patient's body. Thereby cancer cells are identified and suppressed without harming the body's normal cells.^{4,61,62}

In cancer cell therapy using the CAR T cell method, T cells are taken from the patient's blood and then the gene related to a specific receptor called CAR will be inserted into T cells in the laboratory using gene editing methods.^{63–65} These receptors enable T cells to bind to an antigen on the surface of a specific cancer cell.⁶⁶ The type of receptor and antigen may be different according to the type of cancer that is going to be cured. In other words, since different cancers have different antigens, each CAR is designed to bind to a specific cancer antigen.^{63,67} For example, in certain types of leukemia or lymphoma, the cancer cells have an antigen called CD19.⁶⁸ Cardiac cell therapies for these cancers work by targeting the CD19 antigen.^{69–71} In the next step, the modified cells are returned to the patient to destroy the targeted cancer cells. Examples of currently approved CAR T-cell therapies are Kymriah, Yescarta, Tecartus, Brexambi, Abecma, and Carvykti.^{72–74} Although most of these drugs are being used in blood cancer treatment, many other CAR T-cell therapies (and similar

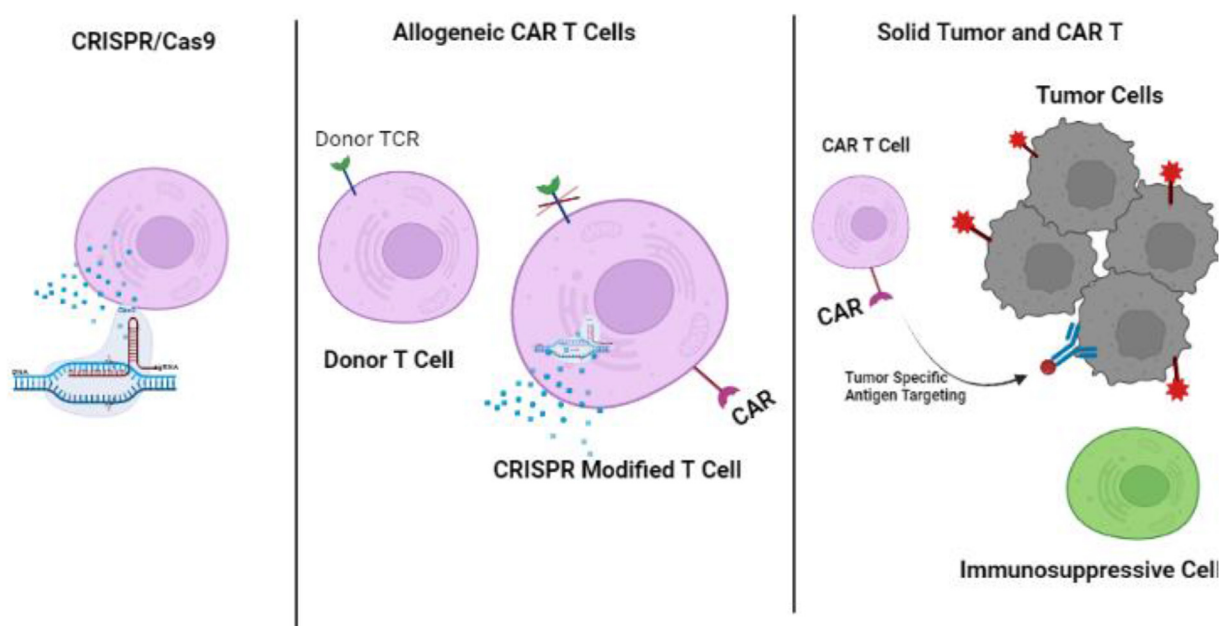


Figure 2 Genetically engineered immune cells with chimeric antigen receptors (CARs) or modified T cell receptors (TCRs) have shown potential as a powerful class of cancer therapeutic strategy.

therapies) are currently undergoing clinical trials.^{75,76} By engineering CAR T cells for allogeneic cell therapy, gene editing can be used to eliminate the risks associated with graft-versus-host disease.⁷⁷ Cas9 mRNA and sgRNA encapsulation into lipid nanoparticles contribute to highly efficient knockdown of T-cell receptor (TCR) in primary human T cells, thus overcoming the shortcomings of conventional delivery methods.^{78,79} Efforts in CRISPR-assisted gene editing technologies are developing as a potential tool to overcome obstacles in CAR-T immunotherapies.^{80,81}

NK cell therapy

NK cells, like T cells, are major players within the immune system.⁸² One of the key differences between these cells is that NK cells are part of the innate immune system, while T cells are a component of the adaptive immune system. NK cells are responsible for immune tolerance and watching the circulation system for abnormal cells such as cancer cells, microbes, or viral-infected cells.⁸³ Despite T cells, NK cells are not antigen-specific and they can basically recognize “non-self” cells, controlled by both activating and inhibitory receptors expressed on their surface. NK cells distinguish cytotoxic particles generated from the foreign cells and remove them.⁸⁴ CRISPR is applicable in knocking down CD38 in essential NK cells for killing cancer cells and preventing graft versus host disease (a condition in which immune cells recognize the patient’s self-cells from foreign cells as a major concern in allogeneic cellular immunotherapy⁸⁵), which may be a common side effect of other allogeneic resistant cell transplants like CAR-T cells.^{86,87} The *ex vivo* extension of NK cells for clinical usage and their *in vivo* short life expectancy and limited capacity to invade benign

tumors are some key issues in this case. Some cancers create transformations that prevent NK cells from working properly.⁸⁸ Comparative to the latest advances in CAR-T cell treatments, CRISPR can be utilized to upgrade NK cell treatments.⁸⁹ For example, like T cells, NKs can be altered precisely to a CAR-NK. Alongside their intrinsic “non-self” cell recognition, the expansion of a CAR implies that they can work both specifically and non-specifically. This phenomenon reduces the probability of cancer cells’ resistance to some treatments. CRISPR can also be utilized to make modifications that increase the life expectancy of some short-life immune cells, improve their cancer-targeting capacity,⁹⁰ and reduce the expression of cancer-related oncogenes. Achieving these goals through CRISPR-Cas9 will help cancer treatments.⁹¹ Other studies incorporate a lipid nanoparticle-based approach to reduce the overexpressed polo-like kinase 1 gene, and a lentivirus-based methodology to target numerous cancer-specific genes.⁹²

NK cell immunotherapy and genetic reconstruction approaches

There are different signaling pathways involved in the insusceptibility of NK cells against tumors.⁹³ Several pathways need to be activated simultaneously to exploit the antitumor potential of NK cell immunotherapy, while other pathways should be suppressed.⁹⁴ The versatility of the CRISPR/Cas9 system fits this requirement perfectly.⁸⁹ CRISPR/Cas9 provides the site-specific integration of desired genes since it can simultaneously edit multiple loci.⁹⁵ Furthermore, CRISPR/Cas9-based gene editing helps better tumor cell detection by NK cells through different pathways.

T cells and the principle of the CRISPR/Cas9 system gene editing system

Three different strategies have been introduced to edit the target genome using CRISPR/Cas9 technique: i) Using sgRNA and plasmid DNA encoding Cas9 protein in a vector³¹; ii) Transferring a combination of Cas9 mRNA and sgRNA, and iii) utilizing ribonucleoprotein (RNP) as a combination of Cas9 protein and sgRNA which is more prevalent compared with the other two methods.⁹⁶ There are minimal off-target effects in the RNP strategy due to its independence to DNA transfer and degradation of the Cas9-gRNA complex. RNP-based transfection represents a rapid, effective, and cost-effective strategy for targeted gene editing. Another RNP advantage is the possibility to design an array of strategies for Cas9-gRNA complex delivery.⁹⁷ Despite the primary delivery methods, the plasmid-based CRISPR/Cas9 system reduces the chance of off-targets in T cells.⁹⁸ The plasmid-based system faces several challenges. After the plasmid entrance to the target cell and nucleus, transcriptional and translational processes will happen to express the encoded proteins. In this way, more time is needed to target the desired gene effectively.⁹⁹ More imperatively, this method results in an irreversible off-target cleavage location.¹⁰⁰ One of the other important challenges based on the plasmid method is the time estimation difficulty due to its multi-factorial nature. In addition, transfection of plasmid DNA probably activates cyclic GMP-AMP synthase process.¹⁰¹ Cas9 mRNA and sgRNA simultaneous transfer to target cells is based on the Cas9/sgRNA complex. Use of mRNAs translated in the cytoplasm is one of the advantages of this method. In addition, mRNA translation reduces the time required for genome modification. Finally, mRNA-based transfection showed a reduced rate of off-target effects compared with the plasmid DNA method. However, this method also has some limitations, like mRNA degradation during transfection preparation or programming.¹⁰² The final form of the CRISPR/Cas9 transfer system is RNP. This approach changes the forms of translation and is the fastest gene editing approach compared with the two other strategies.¹⁰³ Off-target reduction due to the rapid degradation of Cas9, free codon optimization, and promoter selection are the advantages of using RNP.¹⁰⁴ Compared with the plasmid electroporation strategy, which works about 73 h, the RNP process is very fast, and the Cas9 protein is rapidly degraded in the cells within 24 h.¹⁰⁵ Currently, several non-viral nanocarriers like cationic lipid nanoparticles, gold nanoparticles, and imidazole zeolite systems are used to deliver RNP into cells *in vitro*.^{106–108} The CRISPR/Cas9 system has been used in CAR-T cell therapy, recently. Currently, the innovation of T cell transfection by CRISPR/Cas9 transfection using RNP represents a promising approach compared with other transfection methods. T cells are primed by lentiviral and adenoviral vectors to deliver CRISPR components. These transfection methods are ineffective due to gene degradation, poor site-specific attachment, and unprogrammed disruption of undesirable genes.¹⁰⁹ *In vivo* CRISPR/Cas9 transfection shows various circumstances like difficulty in editing validation, immunogenicity, insertional mutagenesis, off-target effects, and safety concerns.¹¹⁰

CRISPR: A game-changer in cancer therapeutics

Adoptive cell therapies for cancer treatment, like tumor-infiltrating lymphocytes (TILs), TCR, CAR-T, and NK, are alluring over conventional medications such as chemotherapy and radiotherapy. They are more focused on cancerous tissues and the depletion of solid tumor cells.¹¹¹ All cell-based immunotherapies include expanding the immune cells *ex vivo* and transplanting them into patients' bodies to fight cancer cells. These adoptive cell therapies can be autologous (separated from the patient) or allogeneic (separated from another donor).¹¹² Whereas all adoptive cell therapies have different obstacles during their clinical usage, CRISPR has been under attention for adoptive cell therapy-based cancer treatment. In this review, different cellular immunotherapies in cancer treatment, CRISPR applications in immunotherapy, and CRISPR-specific cancer targeting have been investigated.

TIL and TCR cell therapies

T cells, frequently called T lymphocytes, have a few cell subsets and are a key component of the adaptive immune system.¹¹³ TILs are resistant cells that attack tumors to kill cancerous cells.¹¹⁴ Autologous TIL treatment includes TIL extraction from a patient's body, *ex vivo* cell expansion, and cell re-infuse into the patient's body. In this way, more TILs will be accessible to treat cancer.¹¹⁵ Despite the early guarantee of TIL treatment, some limitations around their *ex vivo* development in conjunction with their moderately constrained capacity to remove tumors have been raised.¹¹⁶ TCR treatment can be supposed as an update to TIL treatment. TCR treatment employs T cells that are hereditarily adjusted to specific TCRs on their surface, making them superior for cancer cell recognition.¹¹⁷ TCR treatment seems to be safe, but like TILs, it showed some limitations in its clinical studies.¹¹⁸ The need for specificity in TCR treatment has raised some clinical safety issues due to the down-regulation of major histocompatibility complexes by TCR cells.¹¹⁹ Expression of endogenous TCRs near the transgenic TCR is additionally a key issue. It leads to a competition for signaling components and the arrangement of heterodimers that can cause deadly autoimmunity or graft versus host disease.¹²⁰ Recently by developing CRISPR and T cells, some experiments have become time-consuming, problematic, and costly.¹²¹ Safety concerns related to the utilization of retroviral and lentiviral vectors for gene modifications were a restricting issue.¹²² CRISPR system has driven a restoration of both TIL and TCR cell treatments.¹²³ In TILs, CRISPR can be used to modify a gene that represses T cells called cytokine-actuated SH2. It should be noted that the disturbance of cytokine-actuated SH2 increments the TIL hostility towards tumors.¹²⁴ Within the case of TCR cells, CRISPR can be used to knock in the specified TCR to a particular locus within the T cell genome to increase its expression. It can also be utilized to modify the endogenous TCR gene that can cause graft versus host disease and other undesired diseases. Besides, CRISPR

creates gene editions in exceptionally short timeframes with more safety than viral vectors.¹²³

CAR-T cell therapy could be joined by gene-editing techniques. For example, T cells are altered to a specific CAR on their surface.¹⁰⁹ CAR helps T cells to recognize the antigens delivered by the cancer cells to kill them (Fig. 1).⁶⁶ CAR does not depend on familiar major histocompatibility complexes to recognize and kill cancer cells.¹²⁵ CAR-T cell therapy, both autologous (patient-derived) and allogeneic (non-patient donor), has a few major impediments. For autologous CAR-T cell therapy, getting expansive numbers of T cells from patients that are lymphopenic to other medicines and long timeframes for creating an adequate restorative measurement is challenging.¹²⁶ For allogeneic CAR-T cell therapy, the rejection of donor-derived cells by the immune system, and causing diseases like graft versus host disease is challenging.¹²⁷

A few cancers can escape from CAR-T cells by over-expressing certain proteins, like modified cells passing protein 1 on their surface.¹²⁸ CRISPR-Cas9 gene editing has expanded the safety and viability of CAR-T cell therapies in different ways.¹²⁹ First, CRISPR can be utilized to modify the CAR in a focused safe manner and induce long-term expression of the receptor on the cell surface.¹³⁰ Second, CRISPR edits the genome of CAR-T cells to extend their cancer-killing ability. And finally, it can be used to create changes that minimize CAR-T cell exhaustion and increment their long-term multiplication.¹³¹ CRISPR modifications can be utilized for generating “off-the-shelf” CAR-T cell drugs from induced pluripotent stem cells, reducing limitations, and minimizing the safety issues.¹³²

Differences between CAR-T and TCR treatments

The most important difference between CAR-T and TCR treatments is the receptors being utilized and their mode of activity. Although they sound comparative, CAR-T and TCR work differently. TCRs are naturally occurring or negligibly non-specific altered receptors, which rely on major histocompatibility complex proteins. In contrast, CARs are modified receptors that are designed to recognize specific cancer antigens.¹³³ Unlike TCRs, which can recognize extracellular and intracellular components, CARs can only detect extracellular cancer cell materials. It shows that the variety of CAR-T’s target components is less than TCR. CAR-T cells require more antigens compared with TCR cells to be activated. Like other cell therapy techniques, CAR-T cells become “exhausted”, a condition in which their adequacy is reduced.¹³⁴

Current outlook for CRISPR cancer treatment

The field of CRISPR cancer treatment is fast developing with a lot of exciting proof-of-concept data, pre-clinical results being distributed routinely, and numerous modern trials starting during time. In this segment, the current state of CRISPR cancer therapy, the types of cancers being treated,

pre-clinical studies, clinical trials, and FDA-approved treatments will be discussed.

Liquid versus solid tumors

Hematological/non-solid/blood cancers or liquid tumors, like leukemia, lymphoma, and myeloma, have different challenges in their therapy methods compared with solid tumors like breast, lung, or brain cancers.¹³⁵ For example, gene-edited cell therapy is more progressed for hematological malignancies rather than solid tumors.¹³⁶ Whereas tumors can be treated surgically, blood cancers cannot be expelled surgically since their cancerous cells are circulating unreservedly within the body.¹³⁷ In some cases, some tumors cannot be surgically expelled. In this regard, choosing the right treatment method is more challenging due to the complex tumor immune microenvironment and its immunosuppressive impacts.¹³⁸ CAR-T cell therapy was developed for blood cancer treatment because T cells are more potent in assaulting circulating cancer cells instead of tumors. TILs can be utilized to target solid tumors.¹³⁹ In combination with all these therapies, CRISPR is being utilized to improve treatments, expand safety, increase efficiency, and reduce costs and time.¹⁴⁰

Current preclinical investigations and clinical trials

Current CRISPR cancer therapy is developing TILs as safe molecules that target cancer cells to treat lymphocytes. It creates off-the-shelf CAR T cell drugs and alters the NK cells for cancer therapy.¹⁴¹ In the current review, CRISPR is widely studied as a tool for cancer immunotherapy. In Table 1, CRISPR-based trials for non-small-cell lung cancer, esophageal cancer, cervical cancer, metastatic gastrointestinal cancer, T- and B-cell malignancies, multiple myeloma, melanoma, and acute myeloid leukemia, are summarized.^{142,143} Intellia Therapeutics, a company working on CAR-T therapy, reported collaboration with ONK Therapeutics to develop a few diverse CRISPR-edited NK cells for cancer therapy. The FDA has granted a fast-track to a CRISPR-mediated TCR cell therapy for acute myeloid leukemia treatment, called NTLA-5001. In preclinical studies, Editas Pharmaceutical has created induced pluripotent stem cell-derived NK cells, utilizing CRISPR-Cas12a to knock in genes that increment the NK cell activity and its tumor-depletion ability.

Long-lasting action time of CRISPR cancer therapeutics

It has been validated that utilization of both gene-edited cell therapy and *in vivo* CRISPR gene editing tools will empower the treatment of different cancers. In this article, the applications of the CRISPR system in parallel with CAR T-cell and NK cell therapies have been reviewed. Recent discoveries on CRISPR/Cas nucleases technology in cancer

Table 1 Clinical trials based on the CAR-T cell therapy in the context of the tumor immunotherapy registered in [ClinicalTrials.gov](https://clinicaltrials.gov) (May 2022; available at <https://clinicaltrials.gov/>).

NCT number	Condition	Participant number	Location	Status
NCT04280133	Hematologic malignancy	60	United States	Recruiting
NCT04892433	CAR-T cell therapy	150	Italy	Recruiting
NCT04657861	Relapse multiple myeloma (MM), refractory MM	36	China	Recruiting
NCT04658004	Acute myeloid leukemia (AML)	36	China	Not yet recruiting
NCT04670055	Relapse MM, refractory MM	50	China	Not yet recruiting
NCT04541368	Relapse MM	50	China	Not yet recruiting
NCT04532203	Acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL)	72	China	recruiting
NCT04532268	ALL, NHL, B cell leukemia/lymphoma	72	China	recruiting
NCT04703686	Lymphoma	78	France	Recruiting
NCT03758417	MM	60	China	Active, not recruiting
NCT03631576	AML	20	China	Recruiting
NCT03937544	B-ALL	10	Malaysia	Recruiting
NCT03068416	B cell leukemia/lymphoma	25	Sweden	Active, not recruiting
NCT04723901	B-ALL	20	China	Recruiting
NCT04723914	B cell lymphoma	20	China	Recruiting
NCT03684889	Leukemia or lymphoma	16	USA	Active, not recruiting
NCT04697940	NHL	30	China	Recruiting
NCT04581473	Gastric and pancreatic cancers	102	China	Recruiting
NCT03525782	Non-small-cell lung cancer	60	China	Recruiting
NCT04010877	AML	10	China	Recruiting
NCT04499339	MM	38	Germany	Recruiting
NCT04429438	B cell lymphoma	11	China	Recruiting
NCT04404660	B-ALL	185	Germany	Recruiting
NCT03916679	Ovarian cancer	20	China	Recruiting
NCT04097301	AML and MM	58	Italy	Recruiting
NCT03356782	Sarcoma	20	China	Recruiting
NCT02132624	B-ALL	15	Sweden	Completed
NCT03767751	MM	80	China	Recruiting
NCT03289455	B-ALL	23	UK	Completed
NCT03288493	MM	220	USA	Recruiting
NCT04718883	Mantle cell lymphoma (MCL)	59	China	Recruiting
NCT04010877	AML	10	China	Recruiting
NCT04148430	B-ALL and B-NHL	90	USA	Recruiting
NCT04484012	MCL	36	USA	Recruiting
NCT04268706	Hodgkin lymphoma (HL)	94	USA	Recruiting
NCT03373071	ALL and NHL	32	Italy	Recruiting
NCT03373097	Neuroblastoma	42	Italy	Recruiting
NCT04653649	HL	30	Spain	Recruiting
NCT04429451	Solid tumors	100	China	Recruiting
NCT00924326	B cell lymphoma	43	USA	Active, not recruiting
NCT04206943	ALL, NHL	24	Turkey	Recruiting
NCT04257578	B cell lymphoma	20	USA	Recruiting
NCT01475058	B cell lymphoma	1	USA	Completed
NCT01583686	Solid tumors	15	USA	Terminated
NCT01218867	Melanoma, renal cancers	24	USA	Terminated
NCT04553393	NHL	80	China	Recruiting
NCT02744287	Pancreatic and prostate cancer	151	USA	Recruiting
NCT03173417	Leukemia	177	China	Completed
NCT04340167	ALL	100	China	Recruiting
NCT03097770	B cell leukemia or lymphoma	100	China	Completed
NCT03706326	Esophageal cancer	20	China	Recruiting
NCT04186520	NHL, MCL	32	USA	Recruiting
NCT04648475	B cell leukemia/lymphoma	40	China	Recruiting
NCT04571138	B cell leukemia/lymphoma	42	USA	Recruiting

(continued on next page)

Table 1 (continued)

NCT number	Condition	Participant number	Location	Status
NCT02028455	Acute leukemia	167	USA	Active, not recruiting
NCT03467256	B-ALL	18	Russian	Active, not recruiting
NCT04544592	B-ALL and B-NHL	50	USA	Recruiting
NCT03765177	ALL, NHL	60	Canada	Recruiting
NCT03448978	MM	30	USA	Recruiting
NCT03573700	ALL	35	USA	Recruiting
NCT02744287	Pancreatic and prostate cancer	151	USA	Recruiting
NCT04029038	B cell leukemia/lymphoma	30	USA	Not yet recruiting
NCT04649983	B cell leukemia/lymphoma	40	China	Recruiting
NCT04846439	Acute leukemia	20	China	Recruiting
NCT01454596	Brain tumors	18	USA	Completed
NCT04206943	ALL, NHL	24	Turkey	Recruiting
NCT03125577	B cell malignancies	100	China	Recruiting
NCT02535364	B-ALL	82	USA	Terminated
NCT02445248	Diffuse large B-cell lymphoma (DLBCL)	115	USA	Active, not recruiting
NCT04836507	Adult large B cell lymphoma	91	South Korea	Recruiting
NCT03954106	DLBCL	25	USA	Terminated
NCT03941626	Solid tumors	50	China	Recruiting
NCT02772198	B-ALL, B-NHL	300	Israel	Recruiting
NCT04787263	ALL, DLBCL, promyelocytic leukemia	32	Italy	Recruiting
NCT02992834	B cell lymphoma	10	China	Not yet recruiting
NCT03938987	NHL, ALL	63	Canada	Recruiting
NCT03971799	AML	34	USA	Recruiting
NCT03676504	ALL, NHL, CLL, DLBCL, follicular lymphoma, MCL	48	Germany	Recruiting
NCT04077866	Glioblastoma	40	China	Recruiting
NCT03076437	AML, CLL	28	China	Completed
NCT04133636	MM	120	USA	Recruiting
NCT02650999	DLBCL, follicular lymphoma, MCL	12	USA	Active, not recruiting
NCT03097770	B cell malignancy	100	China	Completed
NCT04599556	T-ALL, T-NHL, AML	108	China	Recruiting
NCT03706326	Esophageal cancer	20	China	Recruiting
NCT04186520	NHL, MCL	32	USA	Recruiting
NCT04571138	Leukemia/lymphoma	42	USA	Recruiting
NCT03356795	Cervical cancer	20	China	Recruiting
NCT02028455	Acute leukemia	167	USA	Active, not recruiting
NCT03467256	B-ALL	18	Russian	Active, not recruiting
NCT04544592	B-ALL, B-NHL	50	USA	Recruiting
NCT03765177	ALL, NHL	60	Canada	Recruiting
NCT02690545	HL, NHL	40	USA	Recruiting
NCT03448978	MM	30	USA	Recruiting
NCT04083495	T cell lymphoma	20	USA	Recruiting
NCT02414269	Solid tumors	179	USA	Recruiting
NCT03483103	B-NHL	61	USA	Active, not recruiting

therapeutics are getting more attention. For instance, the discovery of the I-C Cascade-Cas3 system leads to large-scale deletion in oncogenes. This is because of Cas3's potential in modifying large part of DNA instead of making double-stranded breaks. CRISPR-based epigenome editing approaches are other significant CRISPR system tools in cancer therapy. This approach can be utilized to switch on tumor-silencing genes or switch off the oncogenes. Based on the current and developing CRISPR innovation tools in cancer treatment, a fast increase in pre-clinical and clinical trials is expected. Due to the fruitful, safe, and efficient progression of clinical trials in CRISPR immunotherapy, it

can be a good advanced candidate for real and applicable cancer therapy in the future.

Conflict of interests

The authors declare no conflict of interests.

References

1. Kłos P, Chlubek D. Plant-derived terpenoids: a promising tool in the fight against melanoma. *Cancers*. 2022;14(3):502.

2. Parihar R, Rivas C, Huynh M, et al. NK cells expressing a chimeric activating receptor eliminate MDSCs and rescue impaired CAR-T cell activity against solid tumors. *Cancer Immunol Res.* 2019;7(3):363–375.
3. Qi C, Gong J, Li J, et al. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. *Nat Med.* 2022;28(6):1189–1198.
4. Mohanty R, Chowdhury CR, Arega S, Sen P, Ganguly P, Ganguly N. CAR T cell therapy: A new era for cancer treatment (Review). *Oncol Rep.* 2019;42(6):2183–2195.
5. Munshi NC, Anderson Jr LD, Shah N, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N Engl J Med.* 2021;384(8):705–716.
6. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet.* 2020;396(10254):839–852.
7. Wang M, Munoz J, Goy A, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. *N Engl J Med.* 2020;382(14):1331–1342.
8. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med.* 2018;378(5):439–448.
9. 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(26):2531–2544.
10. Charrot S, Hallam S. CAR-T cells: future perspectives. *Hemasphere.* 2019;3(2):e188.
11. Lemoine J, Ruella M, Houot R. Born to survive: how cancer cells resist CAR T cell therapy. *J Hematol Oncol.* 2021;14(1):199.
12. Nezhad MS, Abdollahpour-Alitappeh M, Rezaei B, Yazdanifar M, Seifalian AM. Induced pluripotent stem cells (iPSCs) provide a potentially unlimited T cell source for CAR-T cell development and off-the-shelf products. *Pharm Res (N Y).* 2021;38(6):931–945.
13. Singh N, Perazzelli J, Grupp SA, Barrett DM. Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. *Sci Transl Med.* 2016;8(320):320ra3.
14. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* 2021;11(4):69.
15. Gagelmann N, Riecken K, Wolschke C, et al. Development of CAR-T cell therapies for multiple myeloma. *Leukemia.* 2020;34(9):2317–2332.
16. Marofi F, Motavalli R, Safonov VA, et al. CAR T cells in solid tumors: challenges and opportunities. *Stem Cell Res Ther.* 2021;12(1):81.
17. Zhao J, Lin Q, Song Y, Liu D. Universal CARs, universal T cells, and universal CAR T cells. *J Hematol Oncol.* 2018;11(1):132.
18. Azimzadeh M, Mousazadeh M, Jahangiri-Manesh A, Khashayar P, Khashayar P. CRISPR-powered microfluidics in diagnostics: a review of main applications. *Chemosensors.* 2021;10(1):3.
19. Doudna JA. The promise and challenge of therapeutic genome editing. *Nature.* 2020;578(7794):229–236.
20. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduct Targeted Ther.* 2020;5(1):1.
21. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science.* 2014;346(6213):1258096.
22. Akram F, Sahreen S, Aamir F, et al. An insight into modern targeted genome-editing technologies with a special focus on CRISPR/Cas9 and its applications. *Mol Biotechnol.* 2023;65(2):227–242.
23. Mojica FJM, Montoliu L. On the origin of CRISPR-Cas technology: from prokaryotes to mammals. *Trends Microbiol.* 2016;24(10):811–820.
24. Ebbo M, Audonnet S, Grados A, et al. NK cell compartment in the peripheral blood and spleen in adult patients with primary immune thrombocytopenia. *Clin Immunol.* 2017;177:18–28.
25. Selvakumar SC, Preethi KA, Ross K, et al. CRISPR/Cas9 and next generation sequencing in the personalized treatment of Cancer. *Mol Cancer.* 2022;21(1):83.
26. Singh A, Chakraborty D, Maiti S. CRISPR/Cas9: a historical and chemical biology perspective of targeted genome engineering. *Chem Soc Rev.* 2016;45(24):6666–6684.
27. Bhardwaj P, Kant R, Behera SP, Dwivedi GR, Singh R. Next-generation diagnostic with CRISPR/Cas: beyond nucleic acid detection. *Int J Mol Sci.* 2022;23(11):6052.
28. Chen Y, Zhang Y. Application of the CRISPR/Cas9 system to drug resistance in breast cancer. *Adv Sci.* 2018;5(6):1700964.
29. Lee J, Chung JH, Kim HM, Kim DW, Kim H. Designed nucleases for targeted genome editing. *Plant Biotechnol J.* 2016;14(2):448–462.
30. Danner E, Bashir S, Yumlu S, Wurst W, Wefers B, Kühn R. Control of gene editing by manipulation of DNA repair mechanisms. *Mamm Genome.* 2017;28(7):262–274.
31. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv.* 2018;25(1):1234–1257.
32. Ahmar S, Gill RA, Jung KH, et al. Conventional and molecular techniques from simple breeding to speed breeding in crop plants: recent advances and future outlook. *Int J Mol Sci.* 2020;21(7):2590.
33. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet.* 2018;19(12):770–788.
34. Kozovska Z, Rajcaniova S, Munteanu P, Dzacovska S, Demkova L. CRISPR: history and perspectives to the future. *Biomed Pharmacother.* 2021;141:111917.
35. Li L, He ZY, Wei XW, Gao GP, Wei YQ. Challenges in CRISPR/CAS9 delivery: potential roles of nonviral vectors. *Hum Gene Ther.* 2015;26(7):452–462.
36. Glass Z, Lee M, Li Y, Xu Q. Engineering the delivery system for CRISPR-based genome editing. *Trends Biotechnol.* 2018;36(2):173–185.
37. Wang Y, Bruggeman KF, Franks S, et al. Is viral vector gene delivery more effective using biomaterials? *Adv Healthcare Mater.* 2021;10(1):e2001238.
38. Kantor A, McClements ME, MacLaren RE. CRISPR-Cas9 DNA base-editing and prime-editing. *Int J Mol Sci.* 2020;21(17):6240.
39. Rajawat YS, Humbert O, Kiem HP. In-vivo gene therapy with foamy virus vectors. *Viruses.* 2019;11(12):1091.
40. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct Targeted Ther.* 2021;6(1):53.
41. Wang SW, Gao C, Zheng YM, et al. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. *Mol Cancer.* 2022;21(1):57.
42. Irving M, Lanitis E, Migliorini D, Ivics Z, Guedan S. Choosing the right tool for genetic engineering: clinical lessons from chimeric antigen receptor-T cells. *Hum Gene Ther.* 2021;32(19–20):1044–1058.
43. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells — challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med.* 2017;9(9):1183–1197.
44. Keshavarz A, Salehi A, Khosravi S, et al. Recent findings on chimeric antigen receptor (CAR)-engineered immune cell therapy in solid tumors and hematological malignancies. *Stem Cell Res Ther.* 2022;13(1):482.
45. Ragoonanan D, Sheikh IN, Gupta S, et al. The evolution of chimeric antigen receptor T-cell therapy in children, adolescents and young adults with acute lymphoblastic leukemia. *Biomedicines.* 2022;10(9):2286.

46. Maaroufi M. Immunotherapy for Hodgkin lymphoma: from monoclonal antibodies to chimeric antigen receptor T-cell therapy. *Crit Rev Oncol Hematol*. 2023;182:103923.
47. Khoshandam M, Soltaninejad H, Mousazadeh M, Hamidieh AA, Hosseinkhani S. Clinical applications of the CRISPR/Cas9 genome-editing system: delivery options and challenges in precision medicine. *Genes Dis*. 2023;11(1):268–282.
48. Jensen TI, Axelgaard E, Bak RO. Therapeutic gene editing in haematological disorders with CRISPR/Cas9. *Br J Haematol*. 2019;185(5):821–835.
49. Afolabi LO, Afolabi MO, Sani MM, et al. Exploiting the CRISPR-Cas9 gene-editing system for human cancers and immunotherapy. *Clin Transl Immunology*. 2021;10(6):e1286.
50. Wagner DL, Koehl U, Chmielewski M, Scheid C, Striepecke R. Review: sustainable clinical development of CAR-T cells - switching from viral transduction towards CRISPR-Cas gene editing. *Front Immunol*. 2022;13:865424.
51. Bashor CJ, Hilton IB, Bandukwala H, Smith DM, Veisheh O. Engineering the next generation of cell-based therapeutics. *Nat Rev Drug Discov*. 2022;21(9):655–675.
52. Goss PE, Strasser-Weippl K, Lee-Bychkovsky BL, et al. Challenges to effective cancer control in China, India, and Russia. *Lancet Oncol*. 2014;15(5):489–538.
53. Ledford H. Medical research: if depression were cancer. *Nature*. 2014;515(7526):182–184.
54. Hossain MS, Karuniawati H, Jaioun AA, et al. Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. *Cancers*. 2022;14(7):1732.
55. Nowak H, Szwacka DM, Pater M, et al. Holistic approach to the diagnosis and treatment of patients with tumor metastases to the spine. *Cancers*. 2022;14(14):3480.
56. Ding H, Zhang J, Zhang F, et al. The challenge of selecting tumor antigens for chimeric antigen receptor T-cell therapy in ovarian cancer. *Med Oncol*. 2022;39(12):232.
57. Amini L, Silbert SK, Maude SL, et al. Preparing for CAR T cell therapy: patient selection, bridging therapies and lymphodepletion. *Nat Rev Clin Oncol*. 2022;19(5):342–355.
58. Hu Y, Feng J, Gu T, et al. CAR T-cell therapies in China: rapid evolution and a bright future. *Lancet Haematol*. 2022;9(12):e930–e941.
59. Smith AJ, Oertle J, Warren D, Prato D. Chimeric antigen receptor (CAR) T cell therapy for malignant cancers: summary and perspective. *J Cell Immunother*. 2016;2(2):59–68.
60. Samadani AA, Keymoradzadeh A, Shams S, et al. CAR T-cells profiling in carcinogenesis and tumorigenesis: an overview of CAR T-cells cancer therapy. *Int Immunopharm*. 2021;90:107201.
61. Bashar SB, Akhter Z, Saha BC, Himaloy MMI, Kulsum U, Rahman MR. CAR T cell immunotherapy that revolutionary breakthrough in human oncology treatment: a review. *Pharmacol Pharm*. 2022;13(11):483–515.
62. Gunnarsdóttir FB. *Function of Innate Immune Cells in Breast Cancer*. Lund University, Faculty of Medicine; 2021.
63. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med*. 2018;24(5):563–571.
64. Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. *Biomark Res*. 2017;5:22.
65. Levine BL. Performance-enhancing drugs: design and production of redirected chimeric antigen receptor (CAR) T cells. *Cancer Gene Ther*. 2015;22(2):79–84.
66. Benmeharek MR, Karches C, Cadilha B, Lesch S, Endres S, Kobold S. Killing mechanisms of chimeric antigen receptor (CAR) T cells. *Int J Mol Sci*. 2019;20(6):1283.
67. Tahmasebi S, Elahi R, Khosh E, Esmailzadeh A. Programmable and multi-targeted CARs: a new breakthrough in cancer CAR-T cell therapy. *Clin Transl Oncol*. 2021;23(6):1003–1019.
68. Schubert ML, Hückelhoven A, Hoffmann JM, et al. Chimeric antigen receptor T cell therapy targeting CD19-positive leukemia and lymphoma in the context of stem cell transplantation. *Hum Gene Ther*. 2016;27(10):758–771.
69. Mohty M, Gautier J, Malard F, et al. CD19 chimeric antigen receptor-T cells in B-cell leukemia and lymphoma: current status and perspectives. *Leukemia*. 2019;33(12):2767–2778.
70. Wagner DL, Koehl U, Chmielewski M, Scheid C, Striepecke R. Review: sustainable clinical development of CAR-T cells - switching from viral transduction towards CRISPR-Cas gene editing. *Front Immunol*. 2022;13:865424.
71. Gupta A, Gill S. CAR-T cell persistence in the treatment of leukemia and lymphoma. *Leuk Lymphoma*. 2021;62(11):2587–2599.
72. Cheng EL, Cardle IL, Kacherovsky N, et al. Discovery of a transferrin receptor 1-binding aptamer and its application in cancer cell depletion for adoptive T-cell therapy manufacturing. *J Am Chem Soc*. 2022;144(30):13851–13864.
73. Valiullina AK, Zmievskaya EA, Ganeeva IA, et al. Evaluation of CAR-T cells' cytotoxicity against modified solid tumor cell lines. *Biomedicines*. 2023;11(2):626.
74. Zurowski D, Patel S, Hui D, et al. High-throughput method to analyze the cytotoxicity of CAR-T Cells in a 3D tumor spheroid model using image cytometry. *SLAS Discov*. 2023;28(3):65–72.
75. Santomasso B, Bachier C, Westin J, Rezvani K, Shpall EJ. The other side of CAR T-cell therapy: cytokine release syndrome, neurologic toxicity, and financial burden. *Am Soc Clin Oncol Educ Book*. 2019;39:433–444.
76. Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma. *Nat Rev Clin Oncol*. 2018;15(1):31–46.
77. Wiebking V, Lee CM, Mostrel N, et al. Genome editing of donor-derived T-cells to generate allogeneic chimeric antigen receptor-modified T cells: optimizing $\alpha\beta$ T cell-depleted haploidentical hematopoietic stem cell transplantation. *Haematologica*. 2021;106(3):847–858.
78. Tay LS, Palmer N, Panwala R, Chew WL, Mali P. Translating CRISPR-Cas therapeutics: approaches and challenges. *CRISPR J*. 2020;3(4):253–275.
79. Foldvari M, Chen DW, Nafissi N, Calderon D, Narsineni L, Rafiee A. Non-viral gene therapy: gains and challenges of non-invasive administration methods. *J Contr Release*. 2016;240:165–190.
80. Zhang H, Qin C, An C, et al. Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. *Mol Cancer*. 2021;20(1):126.
81. Biederstädt A, Rezvani K. Engineering the next generation of CAR-NK immunotherapies. *Int J Hematol*. 2021;114(5):554–571.
82. Burrack KS, Hart GT, Hamilton SE. Contributions of natural killer cells to the immune response against *Plasmodium*. *Malar J*. 2019;18(1):321.
83. Sharma P, Kumar P, Sharma R. Natural killer cells - their role in tumour immunosurveillance. *J Clin Diagn Res*. 2017;11(8):BE01–BE05.
84. Marofi F, Rahman HS, Thangavelu L, et al. Renaissance of armored immune effector cells, CAR-NK cells, brings the higher hope for successful cancer therapy. *Stem Cell Res Ther*. 2021;12(1):200.
85. Sung AD, Chao NJ. Concise review: acute graft-versus-host disease: immunobiology, prevention, and treatment. *Stem Cells Transl Med*. 2013;2(1):25–32.
86. Wang L, Chen Y, Liu X, Li Z, Dai X. The application of CRISPR/Cas9 technology for cancer immunotherapy: current status and problems. *Front Oncol*. 2021;11:704999.
87. Liu Z, Shi M, Ren Y, et al. Recent advances and applications of CRISPR-Cas9 in cancer immunotherapy. *Mol Cancer*. 2023;22(1):35.

88. Kennedy PR, Felices M, Miller JS. Challenges to the broad application of allogeneic natural killer cell immunotherapy of cancer. *Stem Cell Res Ther.* 2022;13(1):165.
89. Elmas E, Saljoughian N, de Souza Fernandes Pereira M, et al. CRISPR gene editing of human primary NK and T cells for cancer immunotherapy. *Front Oncol.* 2022;12:834002.
90. Vahidian F, Khosroshahi LM, Akbarzadeh M, et al. The tricks for fighting against cancer using CAR NK cells: a review. *Mol Cell Probes.* 2022;63:101817.
91. Balon K, Sheriff A, Jacków J, Ł Łaczmanski. Targeting cancer with CRISPR/Cas9-based therapy. *Int J Mol Sci.* 2022;23(1):573.
92. Yan J, Kang DD, Turnbull G, Dong Y. Delivery of CRISPR-Cas9 system for screening and editing RNA binding proteins in cancer. *Adv Drug Deliv Rev.* 2022;180:114042.
93. Bald T, Krummel MF, Smyth MJ, Barry KC. The NK cell-cancer cycle: advances and new challenges in NK cell-based immunotherapies. *Nat Immunol.* 2020;21(8):835–847.
94. Zhang W, Zhao Z, Li F. Natural killer cell dysfunction in cancer and new strategies to utilize NK cell potential for cancer immunotherapy. *Mol Immunol.* 2022;144:58–70.
95. Jang HK, Song B, Hwang GH, Bae S. Current trends in gene recovery mediated by the CRISPR-Cas system. *Exp Mol Med.* 2020;52(7):1016–1027.
96. Lyu P, Javidi-Parsijani P, Atala A, Lu B. Delivering Cas9/sgRNA ribonucleoprotein (RNP) by lentiviral capsid-based bio-nanoparticles for efficient 'hit-and-Run' genome editing. *Nucleic Acids Res.* 2019;47(17):e99.
97. Zhang S, Shen J, Li D, Cheng Y. Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. *Theranostics.* 2021;11(2):614–648.
98. Kornete M, Marone R, Jeker LT. Highly efficient and versatile plasmid-based gene editing in primary T cells. *J Immunol.* 2018;200(7):2489–2501.
99. Fujihara Y, Ikawa M. CRISPR/Cas9-based genome editing in mice by single plasmid injection. *Methods Enzymol.* 2014;546:319–336.
100. Naeem M, Majeed S, Hoque MZ, Ahmad I. Latest developed strategies to minimize the off-target effects in CRISPR-Cas-mediated genome editing. *Cells.* 2020;9(7):1608.
101. Xu X, Wan T, Xin H, et al. Delivery of CRISPR/Cas9 for therapeutic genome editing. *J Gene Med.* 2019;21(7):e3107.
102. Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *J Contr Release.* 2017;266:17–26.
103. Yip BH. Recent advances in CRISPR/Cas9 delivery strategies. *Biomolecules.* 2020;10(6):839.
104. Lin Y, Wagner E, Lächelt U. Non-viral delivery of the CRISPR/Cas system: DNA versus RNA versus RNP. *Biomater Sci.* 2022;10(5):1166–1192.
105. Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Res.* 2014;24(6):1012–1019.
106. Wei T, Cheng Q, Min YL, Olson EN, Siegwart DJ. Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nat Commun.* 2020;11(1):3232.
107. Givens BE, Naguib YW, Geary SM, Devor EJ, Salem AK. Nanoparticle-based delivery of CRISPR/Cas9 genome-editing therapeutics. *AAPS J.* 2018;20(6):108.
108. Jahangiri-Manesh A, Mousazadeh M, Taji S, et al. Gold nano-rods for drug and gene delivery: an overview of recent advancements. *Pharmaceutics.* 2022;14(3):664.
109. Dimitri A, Herbst F, Fraietta JA. Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. *Mol Cancer.* 2022;21(1):78.
110. Razeghian E, Nasution MKM, Rahman HS, et al. A deep insight into CRISPR/Cas9 application in CAR-T cell-based tumor immunotherapies. *Stem Cell Res Ther.* 2021;12(1):428.
111. Titov A, Zmievskaya E, Ganeeva I, et al. Adoptive immunotherapy beyond CAR T-cells. *Cancers.* 2021;13(4):743.
112. Koury J, Lucero M, Cato C, et al. Immunotherapies: exploiting the immune system for cancer treatment. *J Immunol Res.* 2018;2018:1–16.
113. Kumar BV, Connors TJ, Farber DL. Human T cell development, localization, and function throughout life. *Immunity.* 2018;48(2):202–213.
114. Jiménez-Reinoso A, Nehme-Álvarez D, Domínguez-Alonso C, Álvarez-Vallina L. Synthetic TILs: engineered tumor-infiltrating lymphocytes with improved therapeutic potential. *Front Oncol.* 2021;10:593848.
115. Mayor P, Starbuck K, Zsiros E. Adoptive cell transfer using autologous tumor infiltrating lymphocytes in gynecologic malignancies. *Gynecol Oncol.* 2018;150(2):361–369.
116. Wu R, Forget MA, Chacon J, et al. Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. *Cancer J.* 2012;18(2):160–175.
117. Zhao L, Cao YJ. Engineered T cell therapy for cancer in the clinic. *Front Immunol.* 2019;10:2250.
118. Morotti M, Albukhari A, Alsaadi A, et al. Promises and challenges of adoptive T-cell therapies for solid tumours. *Br J Cancer.* 2021;124(11):1759–1776.
119. He Q, Jiang X, Zhou X, Weng J. Targeting cancers through TCR-peptide/MHC interactions. *J Hematol Oncol.* 2019;12(1):139.
120. Chandran SS, Klebanoff CA. T cell receptor-based cancer immunotherapy: emerging efficacy and pathways of resistance. *Immunol Rev.* 2019;290(1):127–147.
121. You L, Tong R, Li M, et al. Advancements and obstacles of CRISPR-Cas9 technology in translational research. *Mol Ther Methods Clin Dev.* 2019;13:359–370.
122. Marcucci KT, Jadowsky JK, Hwang WT, et al. Retroviral and lentiviral safety analysis of gene-modified T cell products and infused HIV and oncology patients. *Mol Ther.* 2018;26(1):269–279.
123. Ghaffari S, Khalili N, Rezaei N. CRISPR/Cas9 revitalizes adoptive T-cell therapy for cancer immunotherapy. *J Exp Clin Cancer Res.* 2021;40(1):269.
124. Fix SM, Jazaeri AA, Hwu P. Applications of CRISPR genome editing to advance the next generation of adoptive cell therapies for cancer. *Cancer Discov.* 2021;11(3):560–574.
125. Dhatchinamoorthy K, Colbert JD, Rock KL. Cancer immune evasion through loss of MHC class I antigen presentation. *Front Immunol.* 2021;12:636568.
126. Graham C, Jozwik A, Pepper A, Benjamin R. Allogeneic CAR-T cells: more than ease of access? *Cells.* 2018;7(10):155.
127. Wagner DL, Fritsche E, Pulsipher MA, et al. Immunogenicity of CAR T cells in cancer therapy. *Nat Rev Clin Oncol.* 2021;18(6):379–393.
128. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol.* 2020;20(11):651–668.
129. Salas-Mckee J, Kong W, Gladney WL, et al. CRISPR/Cas9-based genome editing in the era of CAR T cell immunotherapy. *Hum Vaccines Immunother.* 2019;15(5):1126–1132.
130. Schober K, Müller TR, Busch DH. Orthotopic T-cell receptor replacement—an “enabler” for TCR-based therapies. *Cells.* 2020;9(6):1367.
131. Azangou-Khyavy M, Ghasemi M, Khanali J, et al. CRISPR/Cas: from tumor gene editing to T cell-based immunotherapy of cancer. *Front Immunol.* 2020;11:2062.
132. Rezalotfi A, Fritz L, Förster R, Bošnjak B. Challenges of CRISPR-based gene editing in primary T cells. *Int J Mol Sci.* 2022;23(3):1689.
133. Wachsmann TLA, Wouters AK, Remst DFG, et al. Comparing CAR and TCR engineered T cell performance as a function of tumor cell exposure. *Oncol Immunology.* 2022;11(1):2033528.

134. Salter AI, Rajan A, Kennedy JJ, et al. Comparative analysis of TCR and CAR signaling informs CAR designs with superior antigen sensitivity and *in vivo* function. *Sci Signal*. 2021;14(697): eabe2606.
135. Kievit FM, Zhang M. Cancer nanotheranostics: improving imaging and therapy by targeted delivery across biological barriers. *Adv Mater*. 2011;23(36):H217–H247.
136. Chung H, Jung H, Noh JY. Emerging approaches for solid tumor treatment using CAR-T cell therapy. *Int J Mol Sci*. 2021; 22(22):12126.
137. Tohme S, Simmons RL, Tsung A. Surgery for cancer: a trigger for metastases. *Cancer Res*. 2017;77(7):1548–1552.
138. Alieva M, van Rheeën J, Broekman MLD. Potential impact of invasive surgical procedures on primary tumor growth and metastasis. *Clin Exp Metastasis*. 2018;35(4):319–331.
139. Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front Immunol*. 2019;10:128.
140. Uddin F, Rudin CM, Sen T. CRISPR gene therapy: applications, limitations, and implications for the future. *Front Oncol*. 2020;10:1387.
141. Morgan MA, Büning H, Sauer M, Schambach A. Use of cell and genome modification technologies to generate improved "off-the-shelf" CART and CAR NK cells. *Front Immunol*. 2020;11:1965.
142. Mirza Z, Karim S. Advancements in CRISPR/Cas9 technology—focusing on cancer therapeutics and beyond. *Semin Cell Dev Biol*. 2019;96:13–21.
143. Ramezankhani R, Minaei N, Haddadi M, et al. Gene editing technology for improving life quality: a dream coming true? *Clin Genet*. 2021;99(1):67–83.