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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 tum or 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.


## 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

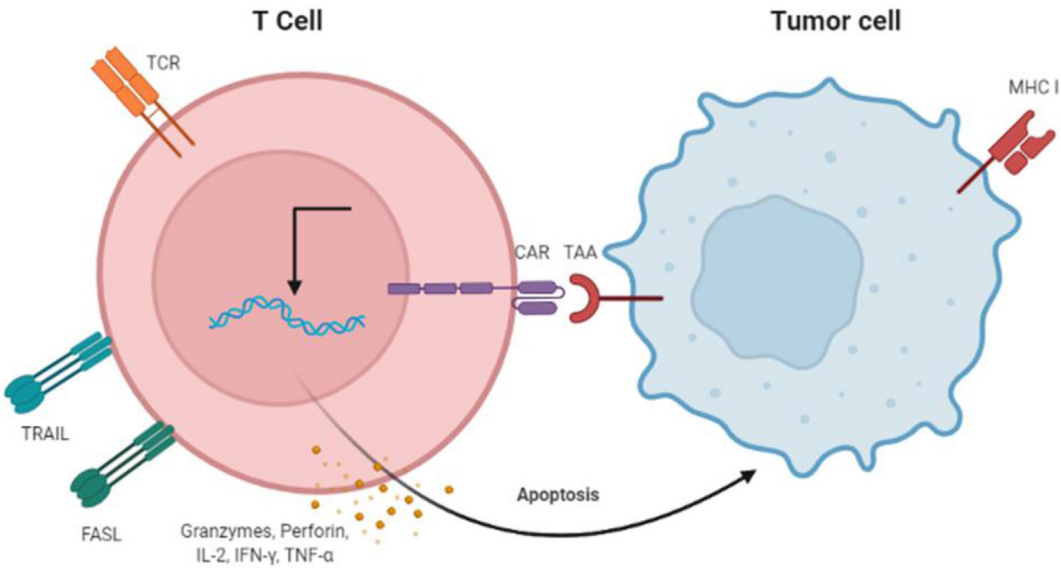


**Figure 1** Schematic view of the interaction between T cells and Tumor cells.

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 corti cells are returned to the patient to destroy the targeted cancer cells. Examples of currently approved CAR T-cell therapies are Kymriah, Yescarta, Tecartus, Breyanzi, 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

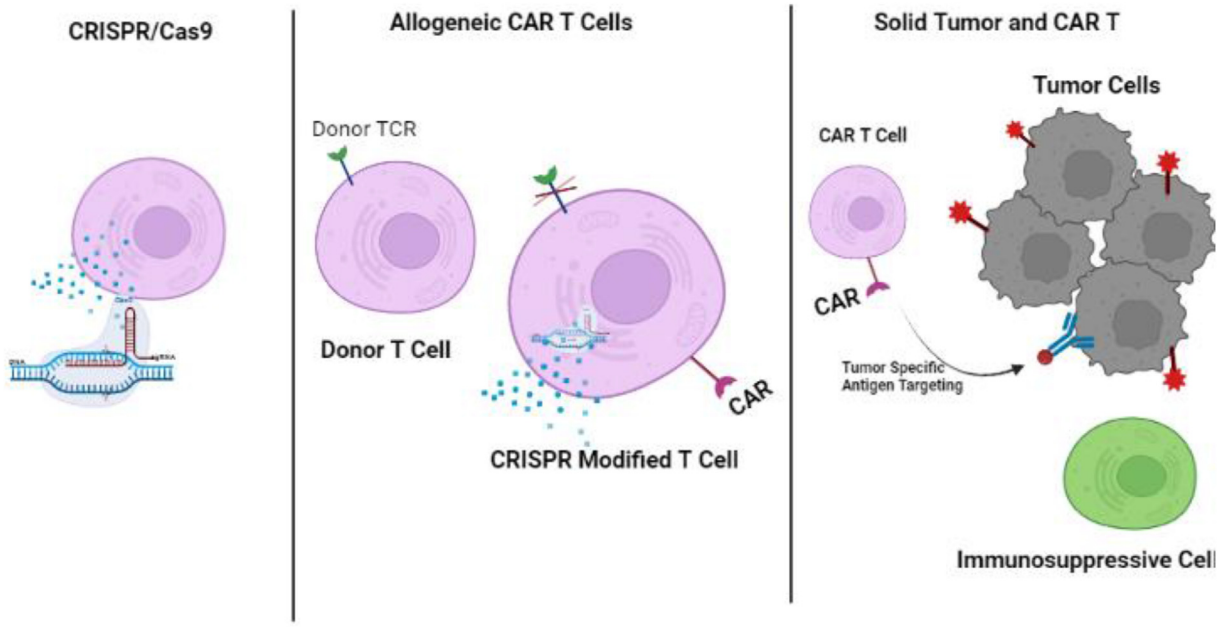


**Figure 2** Genetically engineered immune cells with chimeric antigen receptors (CARs) or modified T cell receptors (TCRs) have shown potential as a powerful class of cancer therapeutic strategy.

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 immunotherapy[85]), 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 vector[31]; 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 multifactorial 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 (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.

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