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# Beyond the Scissors: CRISPR-Cas9's Precision in CAR-T Cell Therapy

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**Abstract**

CRISPR-Cas9 has been revolutionary for editing genes by offering unprecedented precision and versatility in manipulating DNA sequences. This revolutionary tool finds wide applications in medicine, and biotechnology. This paper reviews the remarkable impact of the CRISPR-Cas9 tool on the advancement of one of the newest breakthroughs in cancer immunotherapy: CAR-T cell therapy. An example of CRISPR-Cas9 repercussions in biomedical science is Chimeric antigen receptor (CAR) T cell therapy which represents a groundbreaking advancement in cancer treatment, harnessing the power of the patient's own immune system to fight cancer (Mitra et al. 2023). This paper discusses that CRISPR-Cas9 has the potential to increase both the power and safety of the CAR-T cell therapy via correcting CAR sequence, creating a more robust car that can stay with the patient longer and become more functional as well as the divergent mechanisms of tumour resistance. We further discuss it as a tool for one-day production of "off-the-shelf" CAR-T cells, thus making it less costly and reachable for patients who are a good match with this approach. Additionally, we look into the broader influence of CRISPR-Cas9 in biomedicine such as its participation in the identification of disease mechanisms and the development of new therapies. At last, this article discusses the ethical issues and regulatory aspects of using CRISPR-Cas9 in humans. By giving an all-inclusive synopsis of the current developments and future trends, this review aims to reveal the potent nature of CRISPR-Cas9 in the advancement of CAR-T cell therapy and to push the cancer treatment early detection forward.

**Keywords:** CRISPR-Cas9, CAR-T Cell Therapy, Leukaemia, Enzyme, genetic engineering

## 1. Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a groundbreaking gene-editing tool that works like a tiny pair of scissors for the DNA. While often used interchangeably, CRISPR and CRISPR-Cas9 represent distinct yet interconnected mechanisms in the realm of gene editing. Understanding their subtle differences is crucial for appreciating the vast potential and specific applications of this groundbreaking technology. At its core, CRISPR is a naturally occurring defence mechanism found in bacteria and archaea. It functions as an adaptive immune system, enabling these prokaryotes to avoid invading viruses and plasmids (Barrangou and Marraffini 2014). This defence mechanism comprises a series of short, repetitive DNA sequences interspersed with unique spacer sequences acquired from

previous encounters with foreign genetic elements. Upon a subsequent attack, the CRISPR system transcribes these spacer sequences into RNA molecules, known as CRISPR RNAs (crRNAs). These crRNAs guide Cas proteins, which possess nuclease activity, to recognize and cleave the matching foreign DNA or RNA, thereby neutralising the threat (Ran et al. 2013). In essence, the core distinction between CRISPR and CRISPR-Cas9 lies in their origin and function, whilst CRISPR is prokaryote adaptive immune system, CRISPR-Cas9 is the adaptation of this to generate a biotechnological tool. CRISPR-Cas9 specifically utilises the Cas9 enzyme and a guide RNA (gRNA) for precise gene editing across various organisms (Hsu, Lander, and Zhang 2014).

Prior to CRISPR-Cas9, gene editing relied on older technologies like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). These techniques, while groundbreaking on their own, were often hindered by complexities in design and synthesis, limiting their widespread adoption. For instance, ZFNs, first described in the mid-1990s (Kim et al., 1996), required intricate protein engineering to achieve target specificity, demanding a deep understanding of protein-DNA interactions. Similarly, TALENs, introduced in 2011(Christian et al., 2010; Wood et al., 2011), involved the assembly of repetitive protein modules, a process that could be laborious and time-consuming. CRISPR-Cas9, on the other hand, represents a specific and ingenious biotechnological application derived from the broader CRISPR system. It has revolutionised gene editing by providing scientists with an unprecedented level of precision and efficiency in manipulating DNA sequences across a wide array of organisms. The simplicity and adaptability of this system, first harnessed for gene editing in 2012 (Jinek et al. 2012), propelled it to the forefront of biomedical research and beyond (Wang et al. 2022).

An example of CRISPR-Cas9 repercussion in biomedical science is Chimeric antigen receptor (CAR) T cell therapy which represents a groundbreaking advancement in cancer treatment, harnessing the power of the patient's own immune system to fight cancer (Mitra et al. 2023).

## 2. What Is CRISPR - Cas9

CRISPR allows scientists to precisely cut DNA at a specific location that they want to change, think of it like fixing a typo in a sentence, but on the level of our genetic code. Once the spot is identified, an enzyme, gRNA, is guided by a special molecule that acts like a GPS which finds the exact spot in the DNA and then the enzyme, Cas9 does the cutting. The cell then tries to fix the cut by using the cell's natural repair mechanisms, and that is when scientists can introduce new DNA sequences to change the original instructions (Sledzinski et al. 2021).

CRISPR, was initially discovered in 1987 by Japanese scientist Yoshizumi Ishino and his research team while conducting studies on the bacterium Escherichia coli (E. coli). The discovery happened unexpectedly, as Ishino and his colleagues encountered an unusual pattern of repeated sequences during their cloning experiments (Ishino et al. 1987). However, due to the lack of sufficient DNA sequence data, the function of these arrays remained a mystery.

Though it seems fairly simple, CRISPR actually involves a complex multi-step process (Figure 1). The CRISPR procedure begins once scientists have identified which genomic sequence has to be changed. Scientists often start by identifying specific genes or mutations linked to a disease or condition. This involves studying patients with the condition, analysing family histories, and conducting genetic sequencing to pinpoint the culprit genes. Targeting is the initial step and is essential to the overall effectiveness of gene editing. In this stage, a carefully designed piece of RNA called gRNA is created, which must be precisely designed to bind only to the intended target sequence, hence the gRNA is engineered to recognise and bind to a specific DNA sequence within the target gene. Once the gRNA is designed, it is combined with

an enzyme called Cas9, which acts as a molecular scissor (Jinek et al. 2012).

The Cas9 nuclease is guided by the gRNA to the target DNA sequence. This complex scans the DNA until it finds a sequence that matches the gRNA, known as the protospacer adjacent motif (PAM). Once the PAM is recognized, the Cas9 nuclease binds to the DNA and creates a double-stranded break at the target site. This targeting step in CRISPR is critical because it determines the efficiency and accuracy of the gene editing process (Nishimasu et al. 2014).

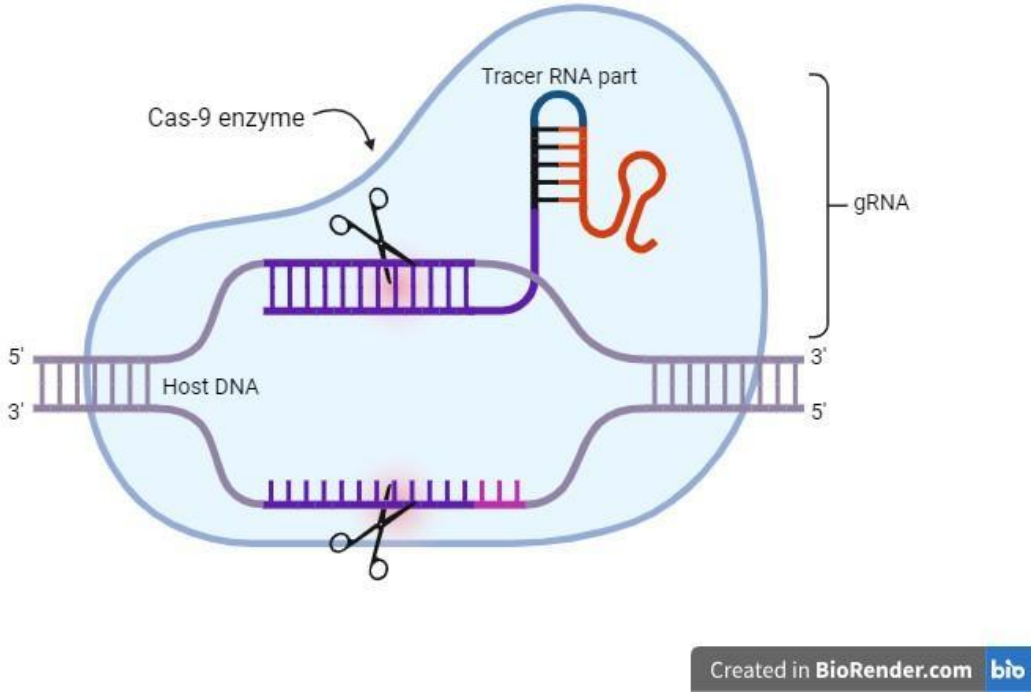


Figure 1. CRISPR-Cas9 system targeting a specific DNA sequence with the guide RNA and Cas9 enzyme and is adapted from Kashtwari (Kashtwari et al. 2022)

The next step is to repair the cut. When the double-strand breaks, this triggers the cell's natural DNA repair mechanisms. The machinery in the cell attempts to repair the break using either of two pathways: non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Figure 2). In NHEJ, DNA ligase IV simply joins the broken ends of the DNA back together. However, this process can introduce small insertions or deletions at the repair site, potentially altering the function of the gene. However, in HDR, the cell uses a template DNA sequence to repair the double-strand break. This template DNA is often provided as a separate piece of DNA and is designed to contain the desired genetic change, such as a gene correction or insertion. The enzymes DNA polymerases which synthesises new DNA to fill in the gaps and DNA ligase I which seals the repaired DNA strands, are also involved in this step (Stinson and Loparo 2021).

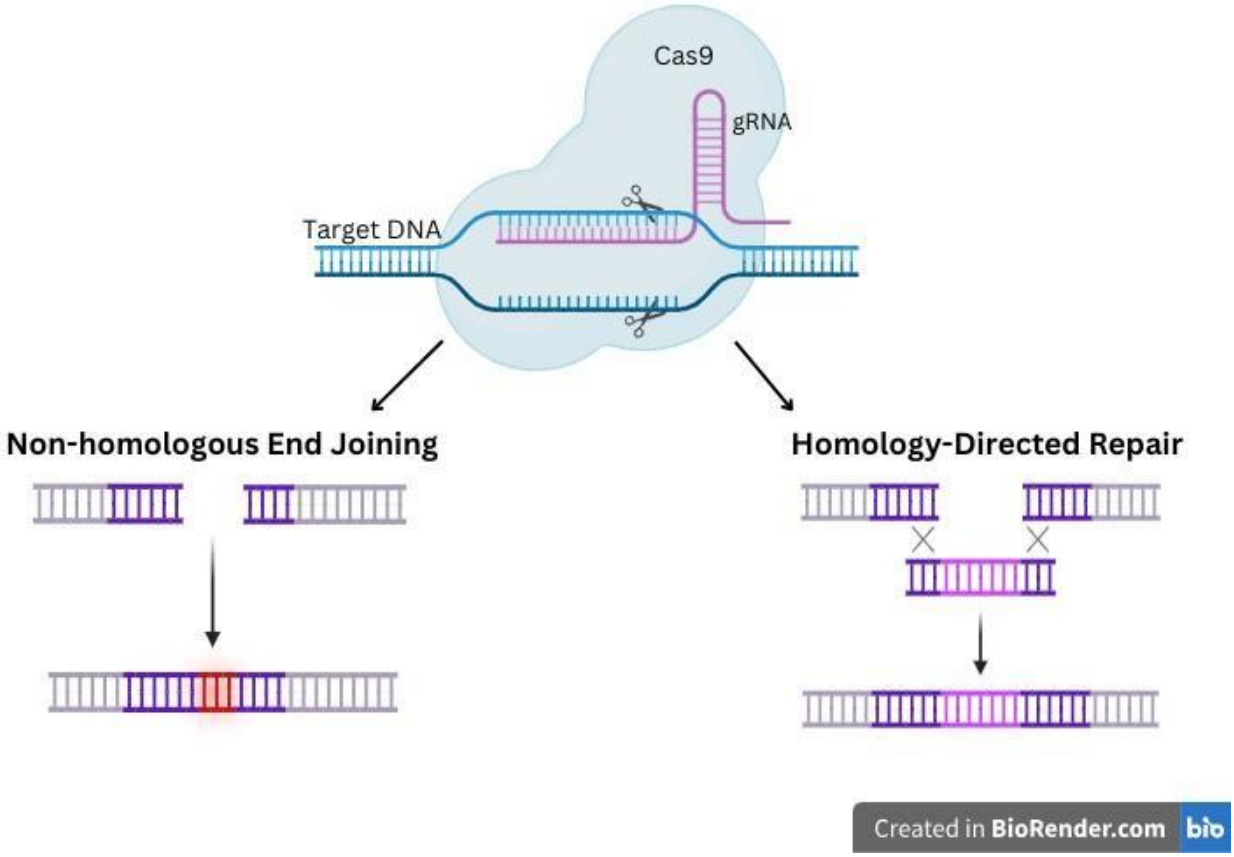


Figure 2. This shows a Cas9 enzyme binding to the target DNA sequence and creating a double stranded break and is adapted from Devi (Devi, Chauhan, and Dhillon 2022)

One of the major concerns with CRISPR-Cas9 gene editing is the off-target effects, a condition where the Cas9 enzyme is mistakenly stuck onto DNA at other places than those intended in the genome. This happens in cases where the gRNA binds to sequences that have slight mismatches, particularly in the regions with low sequence complexity or that contain repetitive elements, e.g. Alu Sequence. These unwanted cuts could lead to deleterious mutations, chromosomal rearrangements and even cell death. From a therapeutic point of view, this is a cause for grave safety concern. With an emphasis on specificity, a number of strategies are under development so as to render CRISPR-Cas9 more specific, reducing off-target editing that includes high-fidelity Cas9 variants and improved gRNA design (Lopes and Prasad 2023).

Scientists have developed new nucleases and Cas9 variants aimed at improving specificity. High-fidelity Cas9, such as SpCas9-HF1 (Kleinstiver et al. 2016b), displays reduced off-target activity while maintaining efficient on-target editing. Other variants, like SaCas9 (Slaymaker et al. 2016), Cas12a (Cpf1) (Zetsche et al. 2017), and Cas12b (Strecker et al. 2019), expand the range of targetable DNA sequences by recognizing different PAM sequences beyond the canonical NGG. These advancements offer improved precision, reducing the risk of unintended genetic alterations.

SpCas9-HF1, a high-fidelity Cas9 variant, enhances precision by reducing unintended edits. Compared to the original Cas9, it has specific changes that weaken interactions with mismatched DNA, thus minimising off-target cuts. While it might be slightly less efficient at the intended target, its superior accuracy makes it invaluable for therapeutic applications like CAR-T cell therapy, where off-target effects could be detrimental (Kleinstiver et al. 2016c).

Another major hurdle in harnessing the power of CRISPR-Cas9 for therapeutic applications is the risk of uncontrolled or persistent DNA cleavage. Once the Cas9 enzyme is introduced into a cell, it can potentially continue cutting the target DNA sequence repeatedly, leading to unintended consequences. This phenomenon can cause chromosomal instability, DNA damage, and even cell death, posing significant challenges for the safe and effective use of CRISPR-Cas9 in clinical settings, particularly in CAR-T cell therapy where persistent editing could compromise treatment efficacy and safety (Lackner et al. 2023).

To address the issue of uncontrolled cutting, scientists are exploring innovative strategies to regulate Cas9 activity temporally and spatially, allowing researchers to initiate and terminate gene editing at specific time points and/or locations, minimising the window for unintended off-target effects. One approach involves the development of inducible systems where Cas9 expression or activity is controlled by external stimuli, such as small molecules or light. For instance, Davis et al. (2015) successfully engineered a Cas9 protein that can be switched on and off using a small molecule demonstrating the feasibility of inducible systems for precise temporal control of editing (Davis et al. 2015). Similarly, other studies have investigated the use of light-activated Cas9 variants, allowing for spatially controlled editing with remarkable precision (Nihongaki et al. 2015), (Yu et al. 2020). Another promising strategy is the development of self-limiting Cas9 variants, which are engineered to degrade or inactivate after a predetermined period or number of cuts. These "suicide" Cas9s offer an inherent safety mechanism, preventing prolonged and potentially harmful editing activity (Hermantara et al. 2024; Jain et al. 2021). These advancements in regulating Cas9 activity represent crucial steps towards ensuring the safe and effective translation of CRISPR-Cas9 technology into therapeutic settings, including CAR-T cell therapy.

In addition to CAR-T cells other therapeutic approaches of CRISPR technologies have been developed. Scientists at University of California, Berkeley are using CRISPR to modify immune cells in people with HIV, aiming to make the cells resistant to the virus. In their study (Li, Holguin, and Burnett 2022), they disrupt the CCR5 gene, which the virus uses to enter cells. Early results from 10 participants show the treatment is safe and the edited cells remain in the body for at least 12 months. While some participants experienced a small decrease in viral load, it was not a significant drop. This trial provides valuable insights into using CRISPR to edit human immune cells and its potential for future HIV therapies, though more research is needed.

## 3. Overview of CAR-T Cell Therapy

CAR-T cell therapy is a revolutionary form of immunotherapy that harnesses the patient's immune system to combat cancer. This highly personalised approach involves engineering the patient's T cells, a type of white blood cell, to express a synthetic receptor known as a CAR, enhancing their ability to recognize and eliminate cancer cells (Varela-Rohena et al. 2008).

This process begins with the extraction of T cells from the patient's blood through a procedure called leukapheresis. In the laboratory, these T cells are genetically engineered to add a CAR gene to them (Figure 3). This gene is usually introduced using viral vectors, carriers that deliver the gene into the T cells. The CAR, which is a synthetic receptor, will provide these T cells with a targeting mechanism so they can identify and bind to a specific protein, or antigen, on the surface of a cancer cell. This specific targeting ensures that the modified T cells will effectively find and destroy tumour cells and have minimal effects on other normal body tissues (Labbé, Vessillier, and Rafiq 2021).

After modification and expansion of the CAR-T cells, rigorous quality control tests follow to ensure safety and efficacy. These tests will confirm that the cells are free from contaminants,

are viable, and can recognise and eliminate cancerous cells. Before the CAR-T cells are infused back into the patient, lymphodepletion may or may not be administered. This is done through chemotherapy to minimise the number of existing T cells, making the environment most conducive for the functioning of the engineered CAR-T cells. After infusion, CAR-T cells circulate in the body while their engineered receptors guide them through the cancer cells. Each time one of the T cells encounters a cancer cell, it binds to the cell antigen and provokes an immune response that causes the killing of the tumour cell (Ceja et al. 2024).

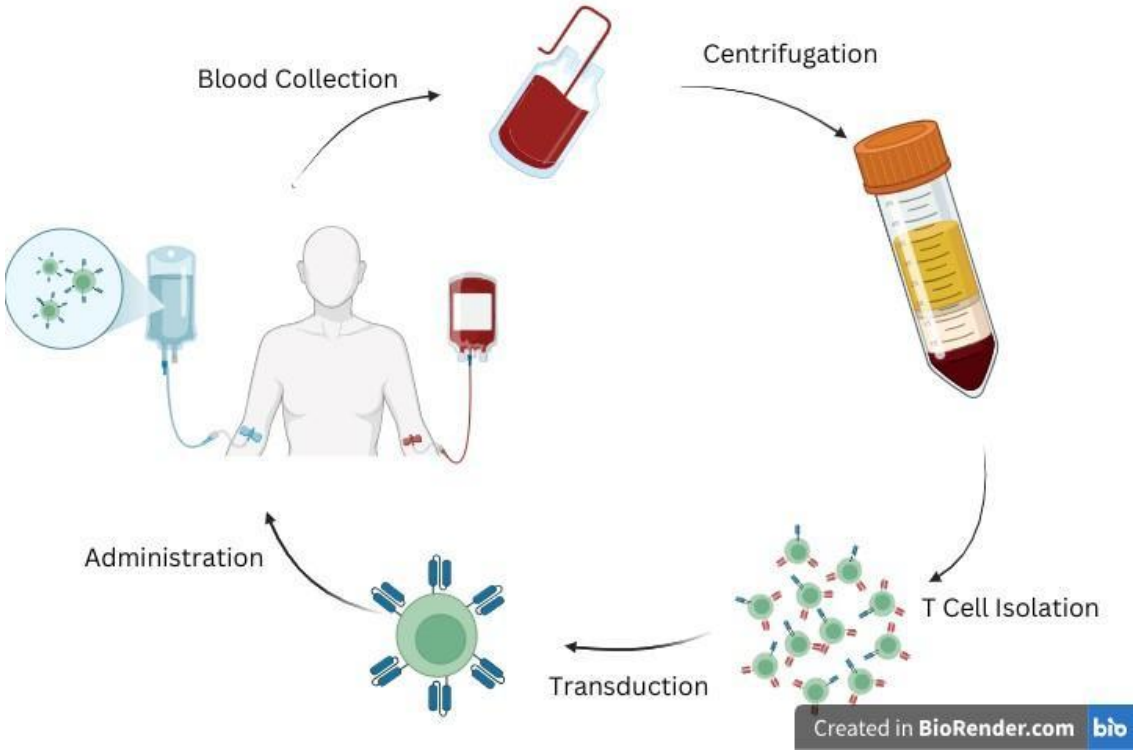


Figure 3. Shows an overview of production of CAR-T cell therapy adapted from Marco (Marco, Monzo, and Ojala 2023)

CAR-T cell treatment has several key benefits when it comes to fighting off cancers. The first aspect is that the therapy in itself is highly personalised in nature, incorporating the use of the patient's immune system to attack and kill the cancerous cells (Rosenberg and Restifo 2015). CAR-T cells were engineered to be highly specific in their recognition and attack against cancer cells while being harmless to healthy cells. This is a very targeted approach that limits the damage to healthy tissues often associated with more traditional cancer treatments (Grupp et al. 2013). CAR-T cell therapy has recently achieved unparalleled success in selected blood cancers with durable remissions, thus offering new hope to patients who have exhausted all options (T. Chen et al. 2024).

CAR-T cell therapy has achieved remarkable success in treating certain blood cancers, such as acute lymphoblastic leukaemia (ALL) and diffuse large B-cell lymphoma (DLBCL). In many cases, it has led to complete remissions, even in patients who had exhausted all other treatment options. This success has been demonstrated the impressive efficacy of CAR-T cells in treating relapsed/refractory ALL (Cappell and Kochenderfer 2023). However, despite its promise, CAR-T cell therapy faces several challenges including relapse, toxicity, and limited applicability to solid tumours.

Some patients experience cancer relapse after CAR-T cell therapy. This can occur due to

several factors, including the loss or downregulation of the target antigen on cancer cells, making them invisible to the CAR-T cells. Another mechanism of relapse is T cell exhaustion, where the CAR-T cells become dysfunctional and lose their ability to effectively kill cancer cells (Shah and Fry 2019). CAR-T cell therapy can cause significant side effects, the most common being cytokine release syndrome (CRS). CRS is a systemic inflammatory response triggered by the rapid activation and proliferation of CAR-T cells. It can cause symptoms such as fever, chills, hypotension, and in severe cases, organ damage. Another potential side effect is neurotoxicity, which can manifest as confusion, seizures, and encephalopathy. The management of these toxicities is a crucial aspect of CAR-T cell therapy (H. Chen et al. 2019). While CAR-T cell therapy has shown great promise in blood cancers, its efficacy in solid tumours has been more limited. This is due to several factors, including the immunosuppressive nature of the tumour microenvironment, the physical barriers that hinder CAR-T cell infiltration into the tumour, and the heterogeneity of antigens expressed on solid tumour cells (Fonkoua et al. 2022).

Despite the remarkable success of CAR-T cell therapy in treating certain blood cancers, its widespread adoption as a first-line treatment is hindered by a significant factor, the cost. These therapies are incredibly expensive, often exceeding hundreds of thousands of dollars per patient (Wu et al. 2023). This high cost is attributed to several factors, including the complex manufacturing process, the personalised nature of the treatment and the substantial research and development investments required to bring these therapies to market (Cliff et al. 2023).

Therefore, CAR-T cell therapy often is reserved for the last line, for which patients have failed other conventional treatments that include chemotherapy or stem cell transplantation. This is particularly true in healthcare systems that either have limited resources or have strict thresholds of cost-effectiveness. For instance, National Institute for Health and Care Excellence (NICE) in the UK initially declined to recommend tisagenlecleucel, a CAR-T cell therapy as a routine National Health Service (NHS) treatment because of high costs, despite impressive efficacy in clinical trials. However, after further negotiation over the price, tisagenlecleucel was approved to be used on selected patients with ALL (NICE, 2024).

However, the severe microenvironment of solid tumours counteracts most of the CAR-T cell functions. This is usually characterised by immune suppressive elements, which either make tumour infiltration a hard task for CAR-T cells or limit their efficiency in killing tumour cells (NCI, 2013). Furthermore, solid tumours are really made of diverse types of cells with different antigen expressions, making it difficult to target all the cancerous cells using a single CAR-T cell therapy (Kailayangiri et al. 2020). Nevertheless, efforts are being made by researchers to develop means of enhancing CAR-T cell therapy against solid tumours. Examples include the development of CARs that target multiple tumour-associated antigens, thus expanding the range of heterogeneity that could be targeted within a tumour cell population (Kyte 2022). Another approach involves "arming" the CAR-T cells with molecules enabling them to overcome the immunosuppressive tumour microenvironment and improve their persistence (Tang et al. 2023). Other approaches also involve a combination of CAR-T cell therapy with other modalities, such as chemotherapy or immune checkpoint inhibitors, to synergistically enhance anti-tumour activity (Alard et al. 2020). Moreover, investigators also are studying the strategies that improve T cell homing to the tumour site, enhancing the capability of CAR-T cells to penetrate the tumour and reach the cancer cells (Chung, Jung, and Noh 2021).

Currently, a number of clinical trials, such as NCT06572956, NCT06508346, and NCT04025216 are in process focusing on investigating the safety and efficacy of CAR-T cell therapy in different kinds of solid tumours, such as glioblastoma, neuroblastoma and lung cancer. These trials also examine various designs of CARs, combination therapies, and

approaches to enhance T-cell functioning in the solid tumour microenvironment. Though still in its infancy in the context of solid tumours, continuous research and clinical trials make CAR-T cell therapy very promising for the future. While scientists continue to develop this promise of treatment, CAR-T cell therapy holds immense promise to become a strong ammunition in fighting a wide array of cancers (Patel et al. 2021).

## 4. CRISPR-Cas9 in CAR-T Cell Therapy

The CRISPR-Cas9 technology revolutionised gene editing with unprecedented accuracy and versatility. CRISPR-Cas9 holds great promise for being able to take CAR-T cell therapy to the next level in both efficacy and safety for this advanced cancer treatment. By using CRISPR-Cas9, researchers will be able to introduce precise modifications in the genetic make-up of T cells aimed at increasing their potential to identify and destroy cancerous cells. That has opened several interesting avenues toward furtherance of CAR-T cell therapy along many important aspects.

The use of CRISPR-Cas9 technology allows the precise insertion of the CAR gene into the DNA of the T cell (Moradi et al. 2024). This has been claimed to allow for improved antigen recognition and T-cell activation and enhances the capability of CAR T cells to recognize and destroy tumour cells (Tao et al. 2024a). First, this significantly improves target specificity of CAR-T cells via improved capability of recognition and binding to cancer cells. This strengthens the activation of T cells, making them even stronger in their attack against tumour cells. It enhances the overall efficacy in the performance of the CAR-T cells by eliminating tumours (Goel et al. 2017). Therefore, this targeted insertion will allow for better antigen recognition, T cell activation, and potency of CAR-T cells while targeting and killing the cancer cells (Sukumaran et al. 2018). Moreover, CRISPR-Cas9 can be used on the CAR itself to optimise its structure and function for better targeting of tumours and activation of T cells. An example includes using CRISPR-Cas9 to create CARs that would be able to recognize multiple antigens, thus perhaps extending the possibilities of using CAR-T cell therapy against a greater range of cancers. The CRISPR-Cas9 technology made this dream of gene editing with unprecedented accuracy and versatility come true. CRISPR-Cas9 has great potential to take CAR-T cell therapy to the next level in both efficacy and safety for the advanced treatment of cancers (Tao et al. 2024b).

Furthermore, CRISPR-Cas9 can also be applied to the CAR itself in order to optimise its structure and function toward better tumour targeting and T cell activation. Using CRISPR-Cas9, for instance, is a way researchers are investigating how to create CARs that recognize multiple antigens and thus potentially broaden the range of applicability of the CAR-T cell therapy (“CRISPR/Cas9 Technology: Towards a New Generation of Improved CAR-T Cells for Anticancer Therapies”).

One of the first clinical trials NCT03399448 of CRISPR-edited T cells was conducted by a team of researchers from the University of Pennsylvania and Tmunity Therapeutics. Using CRISPR, the researchers enhanced the T cells' cancer-fighting ability by disrupting the gene for PD-1, which normally keeps the immune system in check, and to reduce side effects associated with using donor T cells by knocking out the TCR (T-cell receptor). This was safe, the editing T cells could survive and function in the patients bodies for several months. While this study was small and did not show the treatment cured the cancer, it has become an important first step in the development of using CRISPR-edited T cells in cancer therapy.

The other main challenge in CAR-T cell therapy is related to the persistence and functionality of engineered T cells for an extended period of time within a patient's body. Over a long period of time, these cells can become exhausted, losing their effective potential for killing cancerous

cells. On the whole, such exhaustion is driven by the chronic antigen stimulation and immunosuppressive tumour microenvironment that dampens the T cell response. However, this is a difficult task that could be surmounted through the use of CRISPR-Cas9 by adding genetic modifications for persistence and functionality in CAR-T cells. Such engineered T cells are able to bypass exhaustion and continue exerting their anticancer effect (Song et al. 2024).

One such strategy involves the use of CRISPR-Cas9 to knock out genes responsible for driving T cell exhaustion, including a protein called PD-1 that acts as a kind of "off switch" for the T cells. The idea here is to rejuvenate these exhausted T cells allowing them to maintain their cancer-fighting abilities for extended periods. Aside from removing genes responsible for promoting exhaustion, it's also possible to use CRISPR-Cas9 to introduce new genes that improve T cell function and survival. There are, for instance, attempts at inserting genes that favour the generation of T cell memory, so that the engineered T cells could "remember" the cancer cells and respond effectively in an accelerated fashion upon their second encounter. This would presumably confer long-term immunity against recurrence (Wei, Chen, and Wang 2023; Freen-van Heeren 2020).

The manufacturing process for the CAR-T cells is complex and personalised, taking several weeks to make a tailored treatment for each patient. This poses some logistical problems and hence limits this form of treatment from gaining wide accessibility (Abou-el-Enein et al. 2021). This line of thinking follows that this would allow manufactures to create these treatments in advance, unlike the standard cell-modification approach where cells are harvested and genetically altered on a patient-by-patient basis. In this respect, they are employing CRISPR to modify these donor T cells in their clinical trial NCT04035434. They remove some genes that code for the following proteins or protein complexes such as: the TCR to stop any bad reactions and a protein called CD52 which helps the cells live longer in the body. Thus far the results from this trial are encouraging that these edited cells can safely treat a subgroup of patients with B-cell cancers. For example, in a small group of six children with leukaemia, four of them saw their cancer go into remission after receiving this treatment. As a result, scientists have been trying to use CRISPR-Cas9 in producing "off-the-shelf" CAR-T cell therapies, also known as allogeneic CAR-T cells. These donor cells, therefore, are universal and can be produced in advance, which will then be immediately available to a patient rather than the production being individualised (Depil et al. 2020).

Allogeneic CAR-T cells are prepared from healthy donors, not from the patients themselves, and hold great promise for overcoming some of the deficiencies in autologous CAR-T cell therapy. This "off-the-shelf" approach has several potential advantages including increased availability. As allogeneic CAR-T cells can be prepared in advance and are immediately available to any patient without the preparation of personalised T-cells and the waiting period involved. This is even more important in patients with aggressive diseases who do not have the time to wait for personalised CAR-T cell production (“Allogeneic CAR T-Cell Therapy May Provide Alternate Options in ALL” 2022). Another advantage is the low cost, manufacturing allogeneic CAR-T cells could potentially be more cost-effective than producing individualised autologous CAR-T cells. These “off the shelf” CAR-T cells may be tested for rigorous quality control in advance before administration to patients, therefore, consistent quality and potency of the product will be guaranteed.

Allogeneic CAR-T cell therapy faces challenges, primarily the risk of graft-versus-host disease (GvHD) where the healthy tissues of the recipient body get attacked by the donor T-cells (Chuang et al. 2017a). Researchers have employed various strategies to mitigate this risk, including gene editing to eliminate alloreactivity, depletion of alloreactive T-cells, and strategies to induce immune tolerance to allogeneic CAR-T cells (Aparicio, Acebal, and

González-Vallinas 2023; Hu et al. 2021).

CRISPR-Cas9 plays an important role in this strategy, which allows for the removal of genes from the recipient that could provoke an immune response and, therefore, donor cell rejection (Chuang et al. 2017b). The scientists suppress the TCR to avoid GvHD and make the CAR-T cells universally compatible so that they can easily be administered to patients without an extensive search to find an HLA match, therefore making this life-saving therapy at least hypothetically available for wider use. In addition to disrupting the TCR, there is also an added potential of CRISPR-Cas9 to remove other genes which contribute to immune rejection, such as HLA genes (Kang, Li, and Mei 2024).

## 5. Conclusion

CRISPR-Cas9 is the revolutionary gene-editing tool that enables very precise alterations to be made in DNA and, by harnessing this technology, scientists are able to correct genetic defects, create new drugs, and improve CAR-T cell therapy. Very successful in treating blood cancers, CAR-T cell therapy has its challenges, it is very expensive and limited efficiency against solid tumours. CRISPR-Cas9 can further improve CAR-T cell therapy by optimising CAR designs, enhancing persistence in T cells, and developing "off-the-shelf" therapies. As with great challenges come great opportunities, so too does CRISPR-Cas9 have immense potential to revolutionise cancer treatment and improve outcomes for patients.

In healthcare, CRISPR-Cas9 offers hope for treating a wide range of genetic disorders. By its ability to precisely alter or repair faulty genes, it can potentially correct the underlying cause of diseases like sickle cell anaemia and cystic fibrosis. Clinical trials are already underway, and the future looks promising for therapies based on CRISPR-Cas9. This is also proving to be invaluable in drug discovery and development, as it enables scientists to study gene function and identify therapeutic targets more efficiently, by creating precise genetic alterations in cells, researchers can screen for potential drug candidates and assess their effectiveness. This accelerated drug discovery process can lead to the development of more effective and personalised treatments.

Although the recent CRISPR-Cas9 technology is revolutionary, it also has its share of challenges and limitations. Among the major challenges to CRISPR-Cas9 are off-target effects wherein the Cas9 enzyme makes cuts in DNA at other positions than required, with the potential for harmful mutations. Researchers try to overcome this issue by constructing more precise variants of Cas9 and guide RNAs. There are challenges in delivering components of CRISPR efficiently into target cells (Kleinstiver et al. 2016a). It also raises profound ethical questions about alteration of the human gene pool by germline editing and possible unintended consequences. This is a development requiring careful consideration and broad societal consensus. The line further blurs between using CRISPR for therapy and for enhancement, which raises difficult questions about what constitutes "normal" and about how social inequalities might become exacerbated.

This widened use of CRISPR-Cas9 in the clinic will require rigorous testing for its safety and efficiency with regard to assessing risks and ascertaining the benefits in the long run. There will, therefore, be a need to establish tight manufacturing processes and quality control standards that will ensure such therapies are safe and consistent. Lastly, the high cost of development and manufacturing raises concerns over accessibility and affordability for patients; hence it is important to strategize how to ensure equitable access to potentially life-saving treatments.

In conclusion, CRISPR-Cas9 has represented a new horizon in gene modification. It helps us

understand diseases in more detail and create new treatments, including CAR-T cell therapy. The usage of CRISPR-Cas9 on CAR-T cells aims to make this treatment even safer and more efficient by overcoming several challenges such as recurrence and side effects. This technology highlights its importance and has the potential to revolutionise how we treat cancer and other diseases by fixing faulty genes and boosting our immune system. As research continues, we can expect CRISPR-Cas9 to play an even bigger role in improving healthcare and creating a healthier future for everyone.

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**Data sharing statement**

No additional data are available.



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