Review Article

Gene Editing in Cancer Immunotherapy: Mechanisms, Advancements, Limitations and Future Directions

ABSTRACT

Gene editing has emerged as a transformative approach in cancer immunotherapy. Several gene editing tools have been employed for precise modification of the DNA of immune cells, enhancing their ability to target and eliminate cancer cells. This review examines the evolution and applications of key gene-editing tools, such as CRISPR-Cas9, TALENs, ZFNs, and recent innovations like base and prime editing in the field of cancer immunotherapy.

Promising results have been observed in therapies such as CAR-T and tumor-infiltrating lymphocyte (TIL) treatments, which have shown success in cancers like leukemia and lymphoma. These technologies improve immune system function by disrupting checkpoints, boosting cytokine production, and modifying tumor microenvironments.

Significant clinical trials have demonstrated promising outcomes, such as CRISPR-Cas9-engineered T cells targeting refractory cancers, which showed improved efficacy and safety profiles. However, despite these advancements, there have been limitations, including off-target effects, delivery inefficiencies, immunogenicity, and ethical concerns, alongside the high costs that hinder widespread adoption.

Future directions for gene editing in cancer therapy include the integration of AI and machine learning to enhance target accuracy and guide RNA design, as well as novel gene-editing systems such as the I-C Cascade-Cas3, which facilitates large-scale genomic deletions. Furthermore, CRISPR-based epigenome editing holds promise for further advancing cancer therapies. These innovations, combined with optimized delivery methods, are expected to improve the precision, efficacy, and accessibility of gene editing in cancer immunotherapy.

Keywords: Gene editing, Cancer, Immunotherapy, CRISPR-Cas9, Immune cells

1. INTRODUCTION

Cancer Immunotherapy refers to the concept of enhancing the immune system to target and destroy cancer cells. Cancer immunotherapy has been a goal of cancer treatment for over 100 years. In the last few decades, immunotherapy has become an important part of treating some types of cancer (Horgan *et al*., 2022).

Several immunotherapeutic approaches have been employed so far, and these approaches have shown clinical effectiveness in treating advanced cases of cancers like lung cancer, melanoma, kidney cancer, and others. Some of these approaches have included cancer vaccines (Horgan *et al*., 2022), antibody-drug conjugates (ADCs), and immune checkpoint inhibitors. In addition to this, cellular therapies like chimeric antigen receptor (CAR) therapy have also been employed (Khan, 2019; Horgan *et al*., 2022). Immunotherapy is intended to change how the immune system works so it can better

recognize or attack cancer cells (Horgan *et al*., 2022). Unlike traditional treatments (such as chemotherapy), which directly attack cancer cells, cancer immunotherapy indirectly enhances the body's immune response to attack cancer cells (Khan *et al*., 2023). The system has shown significant promise in treating certain tumors, offering hope for long-term remission and fewer side effects (Horgan *et al*., 2022). Traditional immunotherapeutic methods, however, have had limited success because cancer cells often develop ways to avoid immune detection. To address this challenge, various gene therapy techniques have been explored (Khan *et al*., 2023).

Cancer remains one of the leading causes of morbidity and mortality worldwide, presenting a formidable challenge to global health systems (Khan *et al*., 2023). According to the World Health Organization, cancer accounted for nearly 10 million deaths in 2020. In 2022, there were almost 20 million new cases and 9.7 million cancer-related deaths worldwide. By 2040, the number of new cancer cases per year is expected to rise to 29.9 million and the number of cancer-related deaths to 15.3 million (Khan *et al*., 2023). Despite its success in certain cancers, (such as hematologic malignancies and melanoma), immunotherapy faces limitations, including tumor immune evasion, resistance mechanisms, and off-target toxicities (Khan *et al*., 2023). These challenges necessitate the development of innovative strategies to enhance the specificity, efficacy, and safety of immunotherapeutic interventions (Horgan *et al*., 2022).

The advent of gene editing has marked a revolutionary milestone in the field of biomedical research and therapeutic innovation, particularly in cancer immunotherapy (Khan *et al*., 2023). Gene editing technologies, particularly CRISPR-Cas9, transcription activator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs), have revolutionized the field of molecular biology. These molecular tools offer unprecedented precision in manipulating genomic sequences. In cancer immunotherapy, gene editing provides the opportunity to reprogram immune cells, enhance tumor antigen recognition, and overcome immune resistance to combat several types of tumors (Khalil, 2020). For instance, engineered T-cells, such as chimeric antigen receptor (CAR) T-cells, have demonstrated remarkable efficacy clinically in hematologic cancers (Khan *et al*., 2023). In addition, gene editing approaches targeting immune checkpoints have also shown promise in preclinical studies (Khalil *et al*., 2020).

Despite the increasing use of gene editing in cancer immunotherapy, some studies have identified that significant challenges persist in its therapeutic application for cancer (Wang and Doudna, 2023). This is also similar to other fields of application. Some of the identified challenges/limitations present a downside that impedes clinical translation and need to be addressed for future use. A study by Li *et al*., (2020) noted that the dynamic and heterogeneous nature of the tumor microenvironment complicates the design and implementation of gene-edited therapies (Li *et al*., 2020). This study aims to provide a comprehensive review of the emerging advancements in gene editing technologies and their applications in cancer immunotherapy (Khan *et al*., 2023). Furthermore, it will explore key limitations and challenges that must be addressed to realize the full therapeutic potential of gene editing in oncology. This work seeks to contribute to the growing body of knowledge guiding the future of precision medicine in cancer treatment (Molla *et al*., 2021).

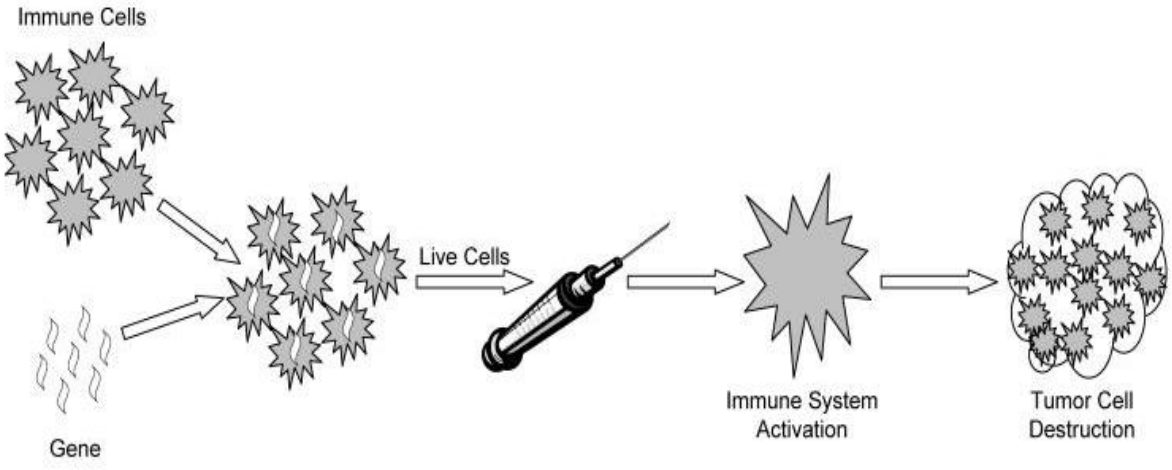

Figure 1: Schematic diagram Pathway representing immunotherapy using altered immune cells (Liu *et al*., 2023)

2. CRISPR-Cas GENE EDITING SYSTEM

The CRISPR-Cas system, which stands for Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated system, has emerged as a revolutionary tool in molecular biology and genetic engineering. It has fundamentally transformed the landscape of gene manipulation thanks to the work of Jennifer Doudna and Emmanuelle Charpentier (Koonin, 2019).

The system was first discovered in the genome of bacteria as an adaptive immune mechanism in bacteria and archaea. Scientists identified it as a critical part of the adaptive immune system of bacteria, functioning by targeting and cleaving foreign DNA from invading phages (Makarova *et al*., 2018). Its modular architecture, which includes short repetitive DNA sequences (CRISPR arrays) and Cas proteins with nuclease activity, has been repurposed for programmable genome editing in eukaryotic systems. There are many variants of the system; however, CRISPR-Cas9 has garnered significant attention due to its high efficiency, specificity, and adaptability in different fields (Makarova *et al*., 2018).

CRISPR-Cas9 is primarily composed of a single-guide RNA (sgRNA) and a Cas9 enzyme, which serves as a restrictive endonuclease. The sgRNA is essentially a nucleotide molecule that directs the Cas9 enzyme to a locus in a complementary sequence of the target DNA. The mechanism of this direction is via Watson-Crick base pairing. This is followed by the nuclease inducing a double-strand break (DSB) at the specified locus (Koonin, 2019). These breaks then activate cellular DNA repair pathways, one of which is known as the non-homologous end joining (NHEJ), which frequently introduces insertions or deletions (indels), or homology-directed repair (HDR), which facilitates precise sequence modifications using a donor DNA template. Scientists take advantage of either of the two repair processes to make modifications in the DNA. The simplicity and versatility of CRISPR are the major reasons for its widespread adoption across disciplines, from functional genomics to therapeutic development (Xia *et al*., 2019; Liu *et al*., 2023).

Recent advancements have expanded the CRISPR system. For example, the CRISPR-Cas12 system has demonstrated unique cleavage properties, including the ability to induce staggered DSBs and cleave single-stranded DNA in a trans-acting manner. These attributes, combined with its broader targeting capabilities due to variations in protospacer adjacent motif (PAM) requirements, make Cas12 a valuable complement to Cas9, particularly for complex genomic engineering and diagnostics (Khalaf *et al*., 2020).

Another pivotal innovation is base editing, which enables precise and irreversible conversion of specific DNA bases (nucleotides) without inducing double-strand breaks or requiring a donor template. By fusing catalytically inactive or nickase Cas variants with deaminase enzymes, base editors directly alter nucleotide sequences (Lin *et al*., 2022). Cytosine base editors (CBEs) convert cytosine (C) to thymine (T), while adenine base editors (ABEs) facilitate the conversion of adenine (A) to guanine (G). This

approach minimizes the risk of off-target effects and unintended genomic rearrangements, offering a safer and more precise alternative for therapeutic applications, such as correcting pathogenic point mutations or modulating oncogenic pathways (Lin *et al*., 2022).

Emerging techniques like prime editing further advance the field by combining a Cas9 nickase with a reverse transcriptase enzyme to enable targeted insertions, deletions, and substitutions without introducing DSBs. This technology provides unparalleled precision and versatility, broadening the range of possible genome modifications and offering solutions to challenges posed by earlier CRISPR iterations (Afolabi *et al*., 2019).

The rapid evolution of CRISPR technologies has encouraged its application in cancer immunotherapy. CRISPR has been used in many clinical and non-clinical settings to enable precise engineering of immune cells, such as chimeric antigen receptor (CAR) T-cells, and to enhance tumor antigen recognition (Afolabi *et al*., 2019). It has also seen adoption in the disruption of immune checkpoint regulators (e.g., PD-1 and CTLA-4). Some studies have reported the use of CRISPR-Cas systems to facilitate the development of synthetic circuits for controlled immune responses (Afolabi *et al*., 2019). Despite their promise, the clinical implementation of these tools necessitates the need to address challenges such as off-target effects, delivery efficiency, and immunogenicity to ensure their safe and effective application in oncological settings (Chen *et al*., 2023).

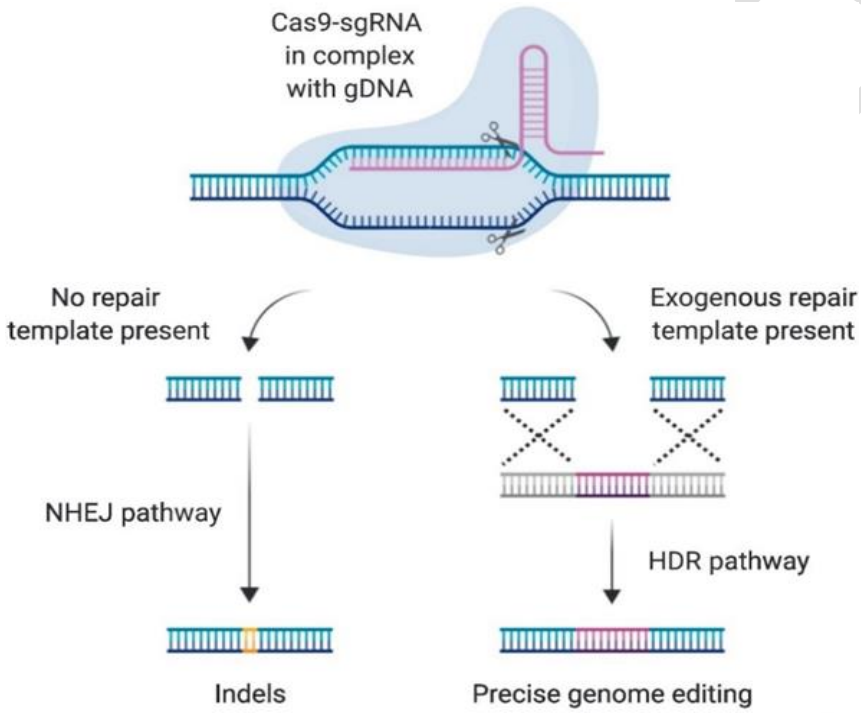

Figure 2: Overview of homology directed repair and non-homologous end joining repair of double-strand breaks generated by Cas9-mediated cleavage in the DNA (van Kampen and van Rooji., 2023)

3. OVERVIEW OF OTHER GENE EDITING TECHNOLOGIES

3.1 Zinc-Finger Nucleases (ZFNs)

Zinc-finger nucleases (ZFNs) **are a gene-editing system that combines** a DNA-binding zinc-finger domain with the FokI endonuclease, which is derived from the bacterium *Flavobacterium okeanokoites*. FokI is a specialized endonuclease responsible for inducing double-strand breaks (DSBs) at specific target sites (Saraswat *et al*., 2023). The zinc-finger domains are the components that recognize specific DNA sequences, enabling precise targeting. However, achieving specificity requires the design of

several zinc-finger motifs, making the process both complex and time-consuming (Saraswat *et al*., 2023).

Despite these challenges, many studies have demonstrated the successful use of ZFNs in therapeutic applications, such as correcting genetic mutations in diseases like sickle cell anemia. ZFNs have also been employed to create genetically modified organisms for research and agricultural purposes (Bhardwaj and Nain, 2021).

3.2 Transcription Activator-Like Effector Nucleases (TALENs)

TALENs are gene editing tools that are similar to the ZFN architecture. However, there are few differences. They consist of transcription activator-like effector (TALE) domains that are linked to a FokI nuclease. TALENs also capable of creating DSBs at specific sites in the genome, but they are more flexible and precise (Harale *et al*., 2022). The TALE domains recognize single nucleotides, making TALENs highly customizable. They've been used in functional genomics, crop engineering, and gene therapy. However, TALENs are quite large, and deliverring them inside cells is tricky. This limits their use compared to CRISPR-based tools (Bhagtaney *et al*., 2023).

3.3 Meganucleases

Meganucleases, or homing endonucleases, are enzymes found in nature. They recognize long DNA sequences, usually 14–40 base pairs long. They have the ability to target rare sites in the DNA and are called rare-cutting endonucleases. These enzymes are very specific and create DSBs at their target sites, which can then be repaired by the cell's natural DNA repair processes (Suzuki *et al*., 2020). While their precision is advantageous, the limited variety of naturally occurring meganucleases restricts their targeting capacity. Engineering new meganucleases to recognize different DNA sequences remains a significant challenge. However, advances in protein engineering hold promise for expanding their applications in gene therapy and synthetic biology (Suzuki *et al*., 2020; Boros *et al*., 2022).

3.4 Antisense Oligonucleotides (ASOs)

Antisense oligonucleotides (ASOs) are short, synthetic DNA or RNA sequences designed to bind to specific RNA molecules. This binding can result in RNA degradation, inhibition of translation, or alteration of splicing patterns. Unlike genome-editing tools, ASOs do not modify the DNA itself but regulate gene expression at the RNA level (Rosenblum *et al*., 2020).

ASOs have demonstrated potential in treating genetic diseases, including Duchenne muscular dystrophy and spinal muscular atrophy. However, their effects are transient and depend on efficient delivery mechanisms, distinguishing them from permanent genome-editing technologies (Suzuki ***et al***., 2020).

4.0 MECHANISMS OF GENE EDITING IN CANCER IMMUNOTHERAPY

Utilizing Gene editing in cancer immunotherapy can enhance the capability of the immune system to fight cancer cells, by indirectly modifying immune cells. Some of the important mechanisms include: T cells engineering for improved tumor recognition, enhancement of tumor-infiltrating lymphocytes (TILs), and overcoming immune checkpoint resistance (Chehelgerdi *et al*., 2024).

4.1. Engineering T Cells: CAR-T Therapy

CAR-T therapy involves using gene editing technologies, such as CRISPR-Cas9, to genetically engineer T-cells to express specific proteins. The cells are first collected from the patient using controlled techniques (Hung *et al*., 2020). They are engineered in vitro and then reintroduced back to the patient. The proteins, known as chimeric antigen receptors (CARs), combine an extracellular tumor-specific antigen recognition domain with an intracellular signaling domain to activate T-cell cytotoxicity. CRISPR-Cas9 plays a pivotal role in this process by enabling the precise insertion of CAR genes into the genome of selected T-cells (Gupte *et al*., 2024).

For example, the CRISPR-Cas9 system is used to knock out genes encoding inhibitory receptors like PD-1 or CTLA-4, known as checkpoints. In cancer, these checkpoints are often exploited by tumors to suppress immune responses. Gene editing removes these inhibitory signals, consequently enhancing the activity and persistence of CAR-T cells (Gupte *et al*., 2024). Additionally, some researchers have

demonstrated that multiplex gene editing allows simultaneous modifications to multiple genes, optimizing CAR-T cells for durability and reducing exhaustion. Another significant advancement is the creation of allogeneic CAR-T cells by knocking out HLA genes in the genome of T-cells. This makes these therapies universally applicable, reducing the risk of immune rejection (Tao *et al*., 2024).

CAR-T therapy has already demonstrated remarkable success in hematologic malignancies during clinical trials. However, engineering efforts are ongoing to address antigen heterogeneity, tumor immune evasion, and other barriers that impede progress in treating solid tumors (Gupte *et al*., 2024).

4.2. Enhancing Tumor-Infiltrating Lymphocytes (TILs)

Tumor-infiltrating lymphocytes are naturally occurring lymphoid cells that can recognize and kill tumor/cancer cells. These leucocytes migrate from the bloodstream toward tumor cells and penetrate them (Gupte *et al*., 2024). They include T cells and B cells. However, their functionality is sometimes impaired by the immunosuppressive tumor microenvironment and the expression of exhaustion markers like TIM-3 and LAG-3. Gene editing is used to knock out TIM-3 and LAG-3 genes in these cells to reverse exhaustion. Removal of these genes or downregulation of exhaustion markers reduces cytotoxic exhaustion, sustaining the cytotoxic activity of tumor-infiltrating lymphocytes (Khan *et al*., 2024).

In some studies, CRISPR-Cas9 has been employed to enhance the capabilities of tumor-infiltrating lymphocytes by modifying the T-cell receptor (TCR) gene in DNA. These engineered T-cells express receptors that better recognize tumor-specific neoantigens, improving specificity and minimizing off-target effects (Zhang *et al*., 2024). Furthermore, gene editing can engineer cytokine signaling pathways, such as IL-2 or IL-15, to boost the proliferation and persistence of tumor-infiltrating lymphocytes in vivo. These enhancements enable TILs to overcome suppressive signals within the tumor microenvironment, mounting a more robust immune response against solid tumors. This has been demonstrated in therapies for melanoma and other cancers (Zhang *et al*., 2024).

4.3 Overcoming Immune Checkpoint Resistance

Immune checkpoints, such as PD-1 and CTLA-4, are regulatory molecules that modulate immune responses to prevent autoimmunity. These checkpoints act as safeguards to prevent immune cells from attacking the body's own cells (Zafar *et al*., 2024). However, tumors exploit these checkpoints to evade immune surveillance. Cancer cells achieve this by upregulating checkpoint ligands (e.g., PD-L1), which mimic the activity of immune checkpoints like PD-1 and CTLA-4. When these ligands bind to corresponding receptors on T-cells, they send inhibitory signals that suppress T-cell activation, preventing further immune responses against the tumor (Zafar *et al*., 2024). Essentially, tumors hijack these checkpoints, allowing cancer to grow and spread undetected (Luo *et al*., 2024).

Gene editing provides a robust strategy for counteracting this resistance by directly targeting checkpoint genes in immune cells (Zhou *et al*., 2024). CRISPR-Cas9 is widely used to knock out PD-1 and CTLA-4 genes in T-cells, delivering the Cas9 component to create double-strand breaks at the targeted genes. This removes inhibitory signals, enhancing T-cell cytotoxicity and allowing sustained activity within the tumor microenvironment. Dual checkpoint editing strategies address redundancies in tumor immune evasion mechanisms, further reducing the tumor's ability to suppress immune responses (Luo *et al*., 2024; Zafar *et al*., 2024).

This mechanism can also be combined with immune checkpoint inhibitors, amplifying their therapeutic effects to overcome checkpoint-resistant tumors. By overcoming these resistance pathways, gene editing significantly enhances cancer immunotherapies, ultimately expanding their applicability to previously untreatable solid tumors (Luo *et al*., 2024).

5. ADVANCEMENTS IN GENE EDITING FOR CANCER IMMUNOTHERAPY

5.1 Successful clinical trials of CRISPR-engineered therapies

5.1.1 CRISPR-Edited T Cells for Solid Tumors

First human study to deploy gene edited T-cell in cancer was a landmark trial conducted in 2020 at the University of Pennsylvania in collaboration with Chinese researchers. The therapeutic potential of PD-1- cells edited with CRISPR-Cas9 was explored for combating malignancies. The trial enrolled three patients (two with metastatic sarcoma and one with advanced myeloma) who had exhausted conventional treatment options (surgery, chemotherapy) (Stadtmauer *et al*., 2020; Kavousinia *et al*., 202)

The gene editing tool used was The CRISPR-Cas9 system. It was employed to knock out the PD-1 gene in T cells. PD-1 is a checkpoint protein that tumors exploit to suppress immune cell activity. T cells were isolated from the blood and CRISPR-Cas9 ribonuclear protein complexes loaded with three sgRNAs were electroporated into the T cells, resulting in gene editing of the TRAC, TRBC1, TRBC2, and PDCD1 (encoding PD-1) loci. Removing PD-1 aimed to enhance T-cell activation and persistence in the tumor microenvironment. After gene editing, t-cells were expanded ex vivo and reinfuse into patients (Bonini *et al*.,2023). To optimize the therapeutic environment, participants were put through a lymphodepleting chemotherapy regimen before receiving the edited cells. The editing process was successful. However, one challenge was ensuring the persistence and efficacy of edited T cells in the immunosuppressive tumor microenvironment. Solid tumors, unlike hematologic malignancies, present physical barriers and a hostile setting that hinder immune cell infiltration and activity (Stadtmauer *et al*., 2020).

The trial demonstrated that CRISPR-Cas9-edited T cells could be administered safely, with no significant off-target effects or severe adverse events. While the clinical efficacy was limited, there were indications of partial anti-tumor activity. These encouraging observations pave the way for future trials to study CRISPR-engineered cancer immunotherapies (Stadtmauer *et al*., 2020).

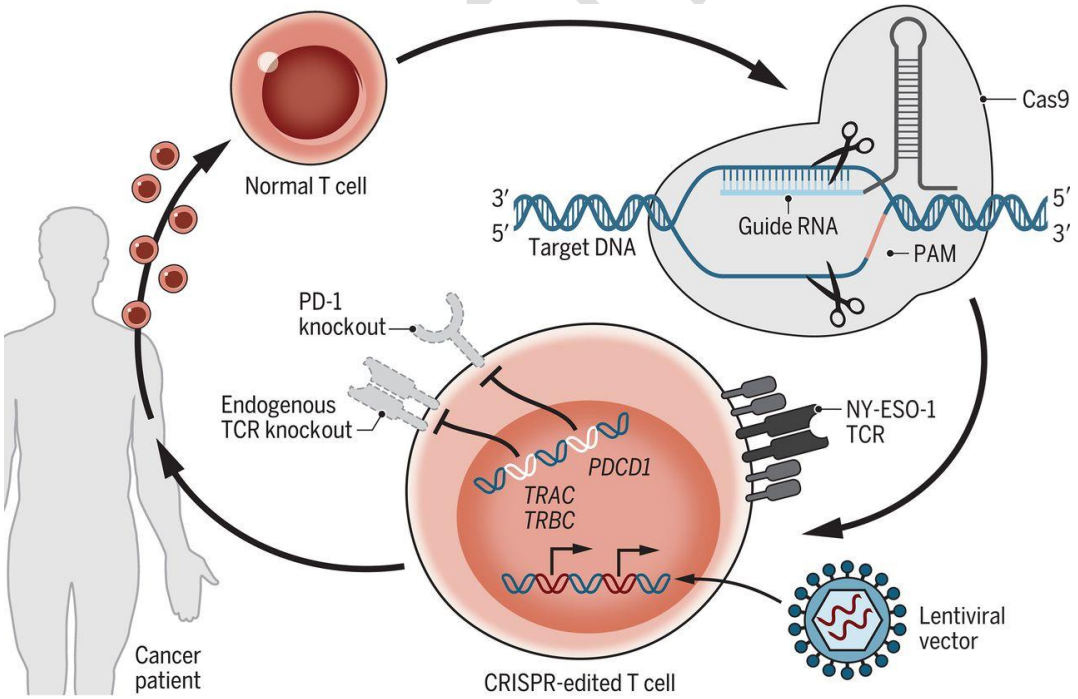

Figure 3: CRISPR-Cas9 engineering of T cells in cancer patients (Stadtmauer *et al*., 2020).

5.1.2 ALPHA (Allogeneic Lymphoma Study of ALLO-501)

This trial was conducted by Allogene Therapeutics. A biotechnology company that is specialized in off-CAR-T therapies (Bonini *et al*.,2023). The trial was focused on ALLO-501. ALLO-501 is an allogeneic CAR-T cell therapy for patients with relapsed or refractory B-cell malignancies, including non-Hodgkin lymphoma. Over 20 patients with advanced-stage B-cell cancers enrolled. These patients had previously shown worsening response to other therapies. Participants included both primary refractory patients and patients with relapses after autologous CAR-T cell therapy (Neelapu *et al*., 2021).

Allogeneic CAR-T cells were engineered using TALEN (Transcription Activator-Like Effector Nucleases) gene-editing tool to knock out endogenous T-cell receptors, preventing graft-versus-host disease (GVHD) (Locke *et al*., 2023). The cells were also engineered to knock out CD52, rendering them resistant to lymphodepleting agents like alemtuzumab, which is used prior to infusion. Additionally, a CAR construct targeting CD19, a B-cell surface antigen, was incorporated. The conductors of the trial reportedly put participants on lymphodepletion with fludarabine, cyclophosphamide, and alemtuzumab, before the infusion of newly edited ALLO-501 cells. The trial reported an overall response rate (ORR) of 75%. While the complete response (CR) rate was reportedly 53%. The therapy demonstrated rapid tumor regression. No cases of GVHD were observed in the study. This trial demonstrated the potential of allogeneic CAR-T therapies to overcome the logistical and financial limitations of personalized CAR-T manufacturing. The study also established a pathway for expanding access to immunotherapy for more patients. Furthermore, Allogene Therapeutics advanced its pipeline, conducting subsequent trials for ALLO-501A (Canichella and de Fabritiis; 2023; Neelapu *et al*., 2021).

5.2 Emerging technologies: Base editing and prime editing in cancer immunotherapy

5.2.1 Base Editing

Base editing was introduced in 2016. It is a precise method for altering single nucleotides without the need for making a double-strand break (DSB) in the DNA or use of a donor DNA template. Instead, the targeted base is chemically modified to create point mutations at targeted genomic site. As a result, base editors can induce precise, small changes in both dividing and post-mitotic cells, bypassing the need for Homology Directed Repair pathways or indel formation (Çerçi, *et al*., 2023)

In cancer immunotherapy, DNA base editors can be used to introduce single point mutations into immune cells. Causing a change in the nucleotide of specific gene sequence such as nucleotides that codes for the chimeric antigen receptor in T-cells. This leads to enhanced immune cells reaction to mutated cancer antigens. For example, the BRAF V600E mutation is a very common mutation in cancers such as melanoma (As well as other cancers like thyroid and colorectal cancer). Base editing can be used to modify T-cells to more effectively recognize and target cancer cells that have this mutation. TCR genes in T-cells can be modified to increase their affinity or specificity for peptide-MHC complexes displaying the mutated BRAF V600E peptide (Nujoom *et al*., 2024)

Notably, a CAR T cell therapy occurred in December 2022, where base-edited T cells were used in a pioneering clinical trial to treat acute lymphoblastic leukemia. The trial resulted in patient remission just 28 days after the infusion of the modified cells. In June 2023, another ground-breaking study published in the New England Journal of Medicine demonstrated that base editing was used to treat aggressive T-cell acute lymphoblastic leukemia in three children. Base editing was then employed to introduce mutations in three specific genes (CD52, CD7, and the β chain of the αβ T-cell receptor) This effectively inactivating these genes to express a chimeric antigen receptor that targets CD7 protein (a protein found on T-cells in acute lymphoblastic leukemia) (Kansal, 2024).

The preliminary results from these studies were promising. However, only certain types of base modifications can be made with base editing. For example, transversion mutations (the conversion of a purine to a pyrimidine base, or vice versa) cannot be achieved. Nonetheless, the high efficiency and ability to introduce multiple edits within a single cell make base editing a promising tool for therapeutic gene editing. (Kantor *et al*., 2020; Harbottle, 2021).

5.2.2 Prime Editing

Prime editing is a next-generation gene-editing tool that expands the versatility of genome modifications by allowing insertions, deletions, and base substitutions with high precision. It employs a prime editing

guide RNA (pegRNA) and a reverse transcriptase enzyme to programmatically rewrite DNA sequences without introducing double-stranded breaks (kantor *et al*., 2020). In cancer immunotherapy, prime editing holds potential for engineering immune cells to target previously intractable mutations within tumor cells or immune regulatory pathways. This approach enhances the functional specificity of T cells and tumor-infiltrating lymphocytes. This allows for paving the way for highly personalized cancer treatments. Prime editing's precise targeting capabilities also mitigate risks associated with genomic instability.

Unlike base editing, the recently developed prime editing technique allows for both base conversions (including transitions and transversions) as well as the generation of small insertions and deletions. Sometimes referred to as a "search and replace" technology. Due to its broad capabilities, prime editing has the potential to correct up to 89% of known genetic variants linked to human diseases (Kantor *et al*., 2020).

Prime editing involves a fusion protein, Cas9 nickase linked to a reverse transcriptase enzyme that identifies the target genomic site and creates a single strand break in the non-target strand. The released 3' DNA strand then binds to the 3' end of the prime-editing guide RNA, which carries the desired edit, and is reverse transcribed by the reverse transcriptase enzyme. After the flap is cleaved, the DNA is ligated, successfully incorporating the edited sequence into the genome.

Several prime editing-based methods have been developed for detecting and treating diseases, and while early proof-of-concept studies show promise, prime editing has not yet been actively used in clinical trials (Kantor *et al*., 2020)

5.2.3 Recent findings

CRISPR-Cas Innovation

CRISPR-Cas12 and Cas13, initially known for their applications in diagnostics, are now being explored for therapeutic editing, including use in cancer immunotherapy. Such innovation has the capacity to offer higher target specificity and reduced immune response risks. Moreso, CRISPR-Cas9 variants, like enhanced Cas9 proteins (e.g., SpRY), have widened the targetable DNA sequences, making regions of the genome that are previously inaccessible to become editable (Hillary and Ceasar, 2023; Leta *et al*., 2024).

Novel Delivery Systems

Effective delivery of gene editing components to target cells remains an important consideration in gene editing. Recent findings show effective results in use of innovatory delivery systems, such as lipid nanoparticles (LNPs) and extracellular vesicles (EVs). The goal being to reduce immune system activation and to enhance efficient delivery. These systems are particularly promising for in-vivo applications in cancer treatment, especially in cases where precise targeting of tumor-associated mutations is necessary (Villiger *et al*., 2024).

Epigenome Editing

Beyond genetic modifications, CRISPR has been adapted for epigenome editing, enabling the modulation of gene expression without altering the underlying DNA sequence. Tools like dCas9 (dead Cas9) have emerged for fused with transcriptional activators or repressors allow for precise control over gene activity. In cancer immunotherapy, this approach has been utilized to upregulate tumor-suppressor genes or downregulate oncogenes, offering a reversible and potentially safer therapeutic strategy. Recent studies highlight its potential in reprogramming tumor microenvironments to enhance the efficacy of immunotherapies (McCutcheon *et al*., 2024).

6. LIMITATIONS AND CHALLENGES

1. Off-target Effects

Off-target effects remain a critical concern in gene-editing technologies. These occur when the editing tool modifies unintended genomic loci of the DNA due to sequence similarities or imperfect targeting

(Bai *et al*., 2020). Such unintended edits can lead to harmful mutations, genomic instability, or oncogene activation in the intended patient. It could sometimes amplify the potential for secondary malignancies. Despite advancements like improved guide RNA designs and high-fidelity variants (e.g., Cas9-HF1), complete elimination of off-target activity remains elusive, especially in complex human genomes (Bai *et al*., 2020)

2. Delivery Challenges

Effective delivery of gene-editing tools into target cells is critical in the success of gene editing for cancer immunotherapy. Current delivery mechanisms, including viral vectors (e.g., use of lentiviruses and Adeno Associated Viruses) and non-viral systems like lipid nanoparticles, often face limitations in tissue specificity, efficiency, and immune compatibility. For T-cell engineering, ex vivo modification provides control over editing, as there is a chance for cross-checking of edited cells before infusion into patients. Contrarily, in-vivo delivery in other applications suffers from low efficiency and potential off-target distribution. Advances in nanotechnology and biomaterial engineering aim to overcome these issues, but scalable and precise delivery remains a challenge (Bai *et al*., 2020)

3. Immunogenicity

The immune system may recognize the introduced gene-editing components, such as Cas proteins or viral vectors, as foreign. This immunogenicity can result in inflammation, reduced efficacy of therapy, or severe immune-related adverse events (Kantor *et al*., 2020). For instance, pre-existing immunity against certain Cas proteins derived from bacterial sources can neutralize the editing tool before it reaches its target. Efforts to mitigate this include engineering immunologically "silent" variants of Cas proteins and using non-viral delivery methods to reduce immune activation (Tao *et al*., 2024)

4. Cost and Accessibility

Gene-editing therapies, particularly CAR-T cell therapies, are expensive, with costs running into hundreds of thousands of dollars per patient. These high costs stem from the personalized nature of treatments, sophisticated manufacturing processes, and the resources required for clinical-grade production and quality assurance. In low- and middle-income countries, these therapies are largely inaccessible, widening the gap in global cancer care. Cost-effective scalable solutions, including universal or off-the-shelf therapies, are being explored to address this disparity (Kantor *et al*., 2020).

5. Ethical Concerns and Regulatory Hurdles

The application of gene editing raises ethical and regulatory challenges, particularly regarding germline editing. There is also the issue of long-term consequences of somatic modifications. Questions about consent, as well as the balance between risks and benefits, and the potential misuse of technologies like CRISPR for non-therapeutic enhancements. One of which is the possibility of making designer babies complicate ethical approval processes (Neelapu *et al*., 2021). Additionally, regulatory frameworks are still catching up with the rapid pace of innovation. Many regions of the world are still less familiar with gene editing tools and have no imminent adoption. This leads to variability in trial approvals and oversight across regions. Harmonized global guidelines and ethical standards are needed to ensure safe and equitable development (Tao *et al*., 2024)

7. FUTURE PERSPECTIVES

7.1 Integration of Artificial Intelligence (AI) and Machine Learning (ML)

Among the most promising developments are the integration of artificial intelligence (AI) and machine learning (ML). most researches argues that such moves are poised to significantly improve the precision and effectiveness of gene-editing techniques (Wang *et al*., 2024). AI and ML can improve gene editing by analyzing vast amounts of genomic data to predict off-target effects, which are one of the major concerns in gene therapy. By using AI-driven algorithms, researchers can identify the most accurate target DNA sites for modification (Khoshandam *et al*., 2023). This will go a long way to minimize unintended effects. Furthermore, Machine learning can help in optimizing the guide RNAs (gRNAs) design processes for CRISPR-based gene editing (Wang *et al*., 2024).

7.2 Personalized Gene-Edited Immunotherapies

The convergence of gene editing with personalized medicine holds tremendous potential for the future of cancer immunotherapy. Approaches like CAR-T cell therapy and tumor-infiltrating lymphocytes (TILs) can be tailored to recognize specific tumor antigens unique to each patient's cancer. This personalized approach maximizes therapeutic efficacy, especially in the context of precision medicine, which adapts treatment strategies based on genetic and immunological profiles (Khoshandam *et al*., 2023; Wang *et al*., 2022).

7.3 Advances in Gene Delivery Systems

One of the critical challenges in gene editing for cancer immunotherapy is the delivery of genetic material to target cells, particularly in in-vivo gene editing. Recent advances in nanoparticle-based delivery systems, such as lipid nanoparticles and polymer-based carriers, are paving the way for safer and more efficient gene delivery. These nanoparticles offer advantages such as reduced immunogenicity, better targeting precision, and the ability to carry larger payloads of genetic material (Jo *et al*., 2023). Improvements in traditional viral vectors, such as adeno-associated viruses (AAVs) and lentiviruses, have also enhanced gene delivery safety and precision (Delbreil *et al*., 2024).

7.4 Discovery of New Gene Editing Systems

Innovative gene-editing systems are emerging that could further advance cancer therapies. The **I-C** Cascade-Cas3 system represents a breakthrough for large-scale gene deletions, which could be applied to oncogenes. Cas3's ability to modify large DNA regions instead of just causing double-stranded breaks opens new possibilities for gene-editing precision in cancer treatment (Sahel *et al*., 2023). Additionally, CRISPR-based epigenome editing approaches are emerging as potential tools for manipulating the epigenetic landscape, offering promising avenues for cancer therapy (Khoshandam *et al*., 2023).

8. CONCLUSION

Gene editing has revolutionized cancer immunotherapy through several tools like CRISPR-Cas9, TALENs, and ZFNs. enabling precise genetic modifications to engineer immune cells such as CAR-T cells and tumor-infiltrating lymphocytes. This review presents some of the Advancements made in this field, including base and prime editing. Furthermore, clinical trials have demonstrated promising results so far, underscoring the potential of gene editing to redefine cancer treatment. However, challenges like off-target effects, immunogenicity, delivery inefficiencies, cost, and ethical concerns remain significant barriers to broader clinical application. Addressing these limitations through innovations in AI-driven precision, advanced delivery systems, and ethical regulatory frameworks is essential for integrating gene editing into mainstream oncology, promising safer, more effective therapies.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

ABBREVIATIONS

AAV - Adeno-Associated Virus

ABEs - Adenine Base Editors

ADCs - Antibody-Drug Conjugates

BRAF - Serine/Threonine-Protein Kinase B-Raf (oncogene)

CAR - Chimeric Antigen Receptor

CAR-T - Chimeric Antigen Receptor T-cell

CBEs - Cytosine Base Editors

CD19 - Cluster of Differentiation 19

CD52 - Cluster of Differentiation 52

CD7 - Cluster of Differentiation 7

CRISPR-Cas9 - Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9

CTLA-4 - Cytotoxic T-Lymphocyte-Associated Protein 4

DNA - Deoxyribonucleic Acid

DSB - Double-Strand Break

GVHD - Graft-versus-Host Disease

HDR - Homology-Directed Repair

IL-15 - Interleukin 15

IL-2 - Interleukin 2

MHC - Major Histocompatibility Complex

NHEJ - Non-Homologous End Joining

PAM - Protospacer Adjacent Motif

PD-1 - Programmed Cell Death Protein 1

PDCD1 - Programmed Cell Death 1

PD-L1 - Programmed Death-Ligand 1

sgRNA - Single Guide RNA

TALE - Transcription Activator-Like Effector

TALENs - Transcription Activator-Like Effector Nucleases

TCR - T-cell Receptor

TILs - Tumor-Infiltrating Lymphocytes

TRBC1 - T-cell Receptor Beta Constant 1

TRBC2 - T-cell Receptor Beta Constant 2

ZFNs - Zinc Finger Nucleases

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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