Enhancing CAR-T Cell Therapy with CRISPR for Blood Cancer Treatment

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Abstract

Blood cancer cases, including lymphoma, leukemia, and multiple myeloma, remain a leading cause of cancer worldwide, with an estimated 1.24 million people being affected annually. Every year, approximately 700,000 people die from blood cancer, highlighting the urgent need for more effective treatments. Currently, chimeric antigen receptor (CAR) T-cell therapy is the most advanced treatment for these cases. CAR-T cell therapy involves genetically modifying patients' T-cells to target and destroy cancer cells. Despite its success, CAR-T cell therapy faces challenges, including side effects like cytokine release syndrome and limited effectiveness against some blood cancers. CRISPR gene editing has the ability to overcome these limitations and strengthen CAR-T cell therapy, offering patients a potential solution by enhancing efficiency and precision in targeting cancer cells. Various types of CRISPR-Cas applications can be used in CAR-T cells to create effective treatment options, such as the creation of "off-the-shelf" allogeneic cells. This review will discuss current issues of blood cancer treatments and how CRISPR can be utilized in next-generation CAR-T cell therapies to improve T-cell engineering, strengthen resistance to tumors, and incorporate safety mechanisms to reduce side effects.

Introduction

Blood cancers affect millions of people worldwide. In the U.S. alone, over 1.24 million people live with blood cancer, and more than 186,000 new cases are diagnosed each year (Bristol Myers Squibb, 2018). These cancers, including leukemia, lymphoma, and myeloma, disrupt blood cell production and can cause serious health issues. Although they account for about 6-10% of all diagnosed cancer cases, they are responsible for 7% of cancer deaths, showing the need for better treatments (Bristol Myers Squibb, 2018; Yale Medicine, 2022).

In recent years, chimeric antigen receptor (CAR) T-cell therapy has become a relatively new and promising treatment that involves modifying T-cells to target and destroy cancer cells more efficiently. This therapy works by extracting a patient's T-cells and then genetically modifying the cell's receptors in a laboratory. The genetically-modified receptor, called CAR, can recognize specific proteins, called antigens, present on cancer cells. These CAR-T cells are infused back into the patient's body to allow for the increased ability to target and fight those cancer cells, as well as other foreign substances (American Cancer Society, 2024)

While CAR-T therapy has shown remarkable success in some cases, especially in patients who did not respond to other treatments, it also presents risks (Phillips, 2023). Side effects like cytokine release syndrome (CRS) and neurotoxicity can occur. CRS occurs when the immune system releases an excess amount of inflammatory molecules, called cytokines, in response to CAR T-cell activation (Chen et al., 2024). This can cause patients to experience symptoms of high fever, low blood pressure, and organ damage, and in some cases, it can be life-threatening. Neurotoxicity, or immune effector cell-associated neurotoxicity syndrome (ICANS), is inflammation of the nervous system. This side effect can lead to difficulty speaking,

seizures, or even fatal cerebral edema, impacting a patient's quality of life and recovery (Chen et al., 2024).

Researchers continue to work toward improving CAR-T cell therapy to help more people fight cancer while trying to limit these negative side effects. Some of these ways include using tools like CRISPR gene editing technologies to enhance T-cell safety and efficiency with hope of reducing unfavorable effects (American Cancer Society, 2024).

CRISPR, which stands for clustered regularly interspaced short palindromic repeats, has the ability to accurately edit DNA at a higher efficiency compared to traditional gene editing methods (Prillaman, 2024). CRISPR is an immune system that was first identified in bacteria and later discovered to be a form of adaptive immunity against viral infections. This developing technology allows for bacteria and archaea to recognize and eliminate invading viruses, as well as prevent mutations by inserting a sequence of viral DNA into their genome to create a genetic memory (Gostimskaya, 2022). The ability of CRISPR to recognize, precisely target, and eliminate the invading viruses, through double stranded breaks in their DNA, has set the foundation for a precise gene editing tool in cells. Scientists have discovered ways to engineer CRISPR for use in mammalian cells. By using specific RNA sequences that can guide CRISPR-associated (Cas) proteins to precise locations in the DNA, researchers have the ability to add, delete, and modify these genes with some accuracy (Prillaman, 2024). While CRISPR is readily used as a tool in research laboratories, its advancements also show great promise in the world of medicine.

CRISPR has the capability to fix genetic mutations that cause some cancers, including blood cancers. This allows for the potential to incorporate CRISPR into CAR-T cell therapy to enhance its abilities to treat patients. Together, CRISPR and CAR-T cell therapy can offer an effective, personalized, and innovative treatment option for targeting the underlying causes of blood cancers. This review focuses on how CRISPR can be used in CAR-T cell therapy to improve the treatment of blood cancers.

MAIN TEXT

The Use of CRISPR in CAR-T Cell Therapy

As advancements in gene editing progress, researchers are finding different approaches to using CRISPR for precision medicine. CRISPR technology has opened new possibilities in the field of immunotherapy by making CAR-T cell therapy a safer and more effective option for treatment. CRISPR being used to optimize the manufacturing process of CAR-T cells makes them more efficient and cost-effective. Scientists can work to improve T cell persistence, reduce toxicity, and increase their ability to evade immune rejection from cancer diseases (Zhang et al., 2021). For example, CRISPR-Cas9 can be used to edit genes within T-cells to either correct mutations or knockout cells that contribute to side effects like CRS or neurotoxicity. By advancing medicine through these technologies, and reducing these immune-related complications, CRISPR-Cas9 will allow for a more effective and sustainable process to target blood cancer cells.

Another approach is to use CRISPR-Cas13, which targets RNA instead of DNA. This method allows for temporary gene silencing without making permanent modifications to the genome (Chen et al., 2024). This is ideal for cancer therapy as it can be used to promote the suppression of genes that drive tumor progression, making it a potentially safer option. Additionally, CRISPR-Cas13 can be used to temporarily turn off genes that control the immune system (Roberts, 2024). This approach would enhance CAR-T cell therapy, making it more efficient in overcoming tumor resistance, lowering the risk of side effects, and making it easier for the cells to fight off tumor resistance.

Studies and Experiments on CRISPR-Modified CAR-T Cells

Several experimental studies have demonstrated the practicality and benefits of using CRISPR in CAR-T cell therapy. Early research showed that gene editing could enhance tumor recognition and improve T cell persistence, leading to prolonged anti-cancer effects (Eyquem et al., 2017). This means that CRISPR modifications allow CAR-T cells to better identify and bind to cancer cells, which ultimately allow for the increase in their effectiveness in targeting tumors.

One way CRISPR helps is by inserting transgenes in T-cells that help boost cytokine production, allowing for an increased immune response against cancerous cells (Song et al., 2024). For example, scientists can use CRISPR to insert the chimeric antigen receptor (CAR) into a specific spot in the T-cell's DNA, such as at the T cell receptor α constant (TRAC) locus, to improve how T-cells operate. These CRISPR-edited T-cells not only have uniform expression of CAR, but they are also able to outperform traditionally generated CAR-T cells when used in a mouse model of leukemia (Eyquem et al., 2017). This further supports the ability of CRISPR to significantly increase tumor-fighting activity and shows the efficiency of CAR-T cell therapy. This method also offers a safer process of engineering T-cells due to the ability of CRISPR to achieve site-specific insertion of the CAR gene.

Another method of enhancing CAR-T cells is to use CRISPR to knock out genes that encode inhibitory receptors, such as PD-1. PD-1 acts as brakes on T-cell activity, making it easier for cancer cells to evade and attack the immune system (Rupp et al., 2017). By editing out PD-1 or similar inhibitory responses, CAR-T cells can persist and function in the aggressive tumor environment, leading to longer-lasting effects.

Recent studies have shown similar findings that have demonstrated improved response rates in patients treated with CRISPR-engineered CAR-T cells (Song et al., 2024). These enhanced CAR-T cells have shown more effective tumor-targeting, prolonged persistence, and reduced cell exhaustion which has led to long-lasting responses in blood cancer patients. By knocking out these inhibitory receptors, CRISPR-engineered CAR-T cells can maintain prolonged activity in patients past remission.

Researchers have also used CRISPR to engineer T-cells that regulate cytokine levels, reducing the risk of unfavorable immune responses (Song et al., 2024). This sort of innovation improves anti-tumor therapy, while preserving the strength and adaptability of CAR-T cells.

Allogeneic CAR-T Cell Therapy Using CRISPR

Another breakthrough involves using CRISPR to develop “off-the-shelf” CAR-T cells, known as allogeneic therapy. This process has been found to increase treatment availability and reduce production costs for families (Memorial Sloan Kettering Cancer Center, 2023). Traditional CAR-T cell therapies are typically derived from a patient’s own T-cells (autologous therapy), which requires extensive processing and manufacturing time. However, instead of creating custom treatments for each patient, scientists use healthy donor T-cells and modify them to be universally compatible. This approach could save time, cut costs, and make treatment readily available.

Multiple clinical trials are currently in the process of testing the efficacy of allogeneic CAR-T cells for various blood cancers. One example is the Phase I trial targeting B-cell maturation antigen (BCMA) in patients with relapsed multiple myeloma (Mailankody et al., 2023). Initial results revealed that over 70% of participants who received an optimal dose of allogeneic CAR-T cells had a partial or complete response rate, supporting the safety and efficacy of this approach. This trial draws attention to the potential of BCMA-targeting allogeneic CAR-T therapy in treating multiple myeloma while addressing challenges such as immune rejection and graft-versus-host disease (GVHD).

One major challenge with allogeneic CAR-T cell therapy is GVHD, a condition in which donor T-cells attack the recipient's healthy tissue, leading to critical complications throughout the body (Lonez & Breman, 2024). Since allogeneic CAR-T cells originate from a donor instead of the patient directly, a goal to keep in mind during their development is to reduce or eliminate the risk of GVHD while maintaining effective anti-tumor activity.

While early trials on allogeneic CAR-T cell therapy are on a promising path toward discovery, there are still challenges to overcome. One major issue is ensuring that the “off-the-shelf” CAR-T cells remain active and productive in patients over time. In addition, reducing risks such as unintended genetic changes or immune rejection is crucial for this approach to become widely used in clinical practice.

As research advances, CRISPR continues to play a central role in improving allogeneic CAR-T cell therapies that will continue to be a focal point in cancer immunotherapy development. One key strategy involves using CRISPR-Cas9 to disrupt genes that contribute to immune evasion and T-cell exhaustion, such as PD-1. By knocking out the PD-1 gene, scientists block signaling pathways that lead to T-cell destruction or dysfunction, which helps to ensure that CAR-T cells, or anti-tumor activity, remain active and effective against tumors (Lonez, 2024). In addition, CRISPR is also used to eliminate endogenous T-cell receptors (TCRs), reducing the risk of GVHD by preventing the engineered T-cells from recognizing and attaching to host tissues. To make this approach more reliable, it can be paired with the knockdown of HLA class I molecules, minimizing the risk of immune rejection. Ongoing studies continue to explore these strategies to improve the safety, reliability, and effectiveness of “off-the-shelf” CAR-T cell therapies, bringing them closer to the development of medicine and clinical applications.

Future Directions and Enhancements

Despite the success of CRISPR-modified CAR-T cell therapies, there is still room for improvement to fully optimize their safety, success, and longevity. Researchers are exploring several methods and technologies to enhance these therapies. One effective method has been to increase T-cell persistence, which reduces exhaustion and improves tumor-targeting specificity (Memorial Sloan Kettering Cancer Center, 2023). Another major area of focus is using CRISPR to delete genes associated with T-cell exhaustion, such as *TOX* and *NR4A*, which would help sustain CAR-T cell activity for longer durations (Wei et al., 2023). Additionally, further advancements in genome editing technologies may allow for more precise modifications which could reduce the risk of off-target effects and thus create safer treatment options.

Beyond CAR-T therapy, CRISPR gives a promising chance at enhancing cancer treatment strategies altogether. Researchers are investigating ways to use CRISPR to modify tumor cells themselves, making the tumors more susceptible to immune system attacks or the effects of chemotherapy (Feng et al., 2024). Other potential applications include engineering immune cells beyond T-cells, such as natural killer cells and macrophages, which could provide an alternative immunotherapy option for patients who do not respond well to CAR-T therapy treatment (Chen et al., 2024). As CRISPR technology advances, its work with CAR-T therapy will likely drive the next generation of cancer treatments, especially blood cancers, offering more effective and accessible options for life.

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