Utilizing CRISPR to Improve the Ability of CAR T-cells to Treat Leukemia

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Abstract

In 2025, there were over 66,000 cases of leukemia in the United States. Common treatments include chemotherapy, targeted drug therapies, and immunotherapies. Despite all of the current treatment options, leukemia has been responsible for over 23,000 deaths in 2025 alone. One form of immunotherapy called CAR T-cell therapy has recently shown great promise as a personalized treatment method for leukemia. However, CAR T-cell therapy can have severe side effects such as infections, potential for secondary cancers, tumor resistance, and autoimmune responses. Clustered regularly interspaced short palindromic repeats (CRISPR) has emerged as a new gene editing technology that has the potential to greatly improve CAR T-cell therapy, helping to reduce these limitations and minimize the side effects. In this review, I will discuss the application of CRISPR to improve the ability of CAR T-cell therapy to treat leukemia.

Introduction

Leukemia is a type of cancer that forms in the blood and bone marrow. It typically occurs due to the bone marrow producing an excessive amount of white blood cells that do not function properly, causing the cells to multiply out of control. White blood cells, also known as leukocytes, are responsible for fighting against infections and will naturally fluctuate in numbers when the body is fighting off an infection. However, in leukemia, the body has a perpetually high white blood cell count, which leads to common symptoms including fatigue, fever, frequent infections, shortness of breath, pale skin, unexplained weight loss, bone and joint pain, swollen lymph nodes, and easy bruising and bleeding (Cleveland Clinic, 2022).

Over 500,000 people are estimated to be living with leukemia in the United States (National Cancer Institute, 2025). In 2025 alone, there were an estimated 66,000 new cases, which makes it 3.3% of all new cancer cases. Leukemia was also responsible for 23,000 deaths in 2025, making it accountable for 3.8% of deaths caused by cancer. Common treatment methods for leukemia include chemotherapy, immunotherapy, targeted drug therapy, radiation, stem cell or bone marrow transplant, and chimeric antigen receptor (CAR) T-cell therapy (Cleveland Clinic, 2022). Chemotherapy uses chemicals to kill leukemia cells or stop them from multiplying, and is currently the most common treatment method for leukemia. Immunotherapy relies on drugs to boost your immune system to help fight leukemia. Targeted therapy uses drugs designed to attack proteins or genes specific to the leukemia cells. Radiation therapy uses X-rays or radiation directed at the location of the leukemia to kill the cancer cells. A stem cell or bone marrow transplant is used in combination with chemo and radiation, and replaces

the cancerous cells with healthy cells from your blood or bone marrow or from a donor. Finally, CAR T-cell therapy uses engineered T-cells specifically programmed to fight the leukemia cells.

CAR T-cell therapy is a novel approach compared to the other leukemia treatment options, as it was first approved by the FDA and used to treat a leukemia patient in 2017 (Ragoonanan et al., 2022). CAR T-cell therapy involves extracting T-cells from the body and altering them in a lab using gene editing technologies to make them express the chimeric antigen receptor. These CAR T-cells are genetically programmed to specifically attack cancer cells by latching on to antigens only present on the cancer cells, and therefore, eradicating them.

Despite there being numerous treatment options available, the 5-year relative survival rate (2015-2021) for leukemia is only 67.8% (National Cancer Institute, 2025). CAR T-cell therapy is an innovative treatment that uses your own immune system and is specifically targeted to the cancer cells, avoiding random damage caused by other treatments, such as chemotherapy. However, CAR T-cell therapy still needs improvement. Advancements to gene editing tools, such as CRISPR technologies, could greatly improve the effectiveness of CAR T-cell therapy and be the future of treating leukemia and bringing the survival rate closer to 100%. In this review, I will discuss the applications of CRISPR in CAR-T cell therapy for leukemia.

Limitations of CAR T-cell Therapy

CAR T-cell therapy has shown great promise in treating leukemia, but it also has many side effects, including some that can be life-threatening. The most common side effect, which affects 54% to 91% of patients, is cytokine release syndrome, which is an inflammatory response caused by the release of a massive amount of cytokines (Hay et al., 2017). Common symptoms include high fever, fast heart rate, low blood pressure, low blood oxygen, rash, headaches, body aches, and, in severe cases, death (Mayo Clinic, 2025). The second most commonly noted symptom that can occur, which affects 20% to 60% of patients, is immune effector cell-associated neurotoxicity syndrome (ICANS) (Rees, 2022). ICANS is also due to the release of cytokines, which cause activation of immune cells, leading to disruption of the blood-brain barrier and neuroinflammation. Some of the symptoms of ICANS are confusion, excessive drowsiness, trouble speaking, fainting, seizures, and brain swelling (Mayo Clinic, 2025). CAR T-cell therapy can also lower your blood cell count, which weakens your immune system, so many patients get infections after receiving treatment. Another challenge that patients face is recurrence because cancer cells can produce multiple types of antigens that allow them to escape the CAR T-cells and multiply over time. Additionally, some people develop secondary cancers, such as blood or bone marrow cancer, within 5 years of receiving CAR

T-cell therapy. Many of these symptoms can be relieved with improvements to CAR T-cell therapy.

CRISPR Technologies

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a novel gene editing tool that has the potential to greatly improve CAR T-cell therapy. CRISPR was first discovered as an immune defense system in bacteria, and scientists have been able to engineer it for use in mammalian cells (Synthego, n.d.). The CRISPR system uses a Cas nuclease, which is an enzyme that can bind to DNA and cause double-stranded breaks. The Cas protein forms a complex with a guide RNA (gRNA) that is complementary to the DNA sequence of interest. This guide RNA can be designed in the lab to target a specific gene or region of the genome. The Cas protein scans the DNA to locate the target provided by the gRNA and then creates the double-strand break. CRISPR gene editing works by taking advantage of the cell's natural DNA repair pathways.

The two most common ones used for gene editing are non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is the quicker, more efficient method than HDR since it is active throughout the whole cell cycle (Yang et al., 2020). It works by adding or removing nucleotides to connect the two broken DNA ends without needing a prior template. Its speed does come with a setback, though, as it is very prone to errors and can lead to a genetic knockout, making the gene non-functional. Scientists can actually use this to their advantage if they want to knock out a mutated gene or induce a mutation in genes, such as those associated with cancer growth. The other common method for gene editing, HDR, is used when the process requires more precision, like inserting a gene or repairing broken genes. This method uses a donor DNA template that locates and precisely repairs the broken DNA, unlike NHEJ, which locates the two broken ends and puts them back together. With HDR, scientists can add a gene into specific parts of the genome, allowing for targeted integration. This method can be used to make CAR T-cells by inserting the chimeric antigen receptor into T-cells.

Use of CRISPR in CAR-T Cell Therapy

While previous ways of creating CAR T-cells, such as TALENS or zinc finger nucleases, were viable methods, CRISPR-Cas9 allows for the CAR to be inserted at precise target regions of the genome, such as insertion into the TRAC locus, with relative ease and few off-target edits (Roberts, 2025). This precise insertion results in uniform CAR expression and enhanced T-cell potency, increasing the safety and efficiency of CAR T-cell therapy (Eyquem et al., 2017).

Not only can CRISPR be used for precise integration of CAR into the TRAC locus, but it can also be used to fix genetic defects or knock out genes in T cells that restrict the cells' ability

to successfully target and kill some cancer cells. This is due to the ability of CRISPR to create edits in multiple genes simultaneously. For example, CRISPR can be used to knock out immune checkpoint receptors that lead to T-cell exhaustion. T-cell exhaustion occurs due to persistent expression of inhibitory receptors, such as PD-1, CTLA-4, LAG3, and TIM3 (Wei et al., 2023). By knocking out these inhibitory receptors, the ligands that activate cell exhaustion, which typically come from cancer cells, can no longer bind. This leads to increased CAR T-cell persistence and the strengthened ability to target cancer cells. Another example involves knocking out Regnase-1, which is an RNase that halts T-cell activation. By performing a genetic knockout of Regnase-1, CAR T-cells no longer become prematurely exhausted, which enhances their persistence and helps sustain their long-term antitumor efficacy.

Another use of CRISPR is for the production of allogeneic CAR T-cells. Unlike traditional CAR T-cells, where the T-cells need to be drawn from the host and then altered in a lab for weeks, allogeneic CAR T-cells are universal, ready-to-go CAR T-cells created by genetically modifying T-cells from healthy donors, which can then be given to patients immediately (Lonez & Breman, 2024). They are held in blood banks and can be taken right off the shelf and given to patients based on the type of CAR T-cell treatment that they need. This approach not only decreases time to treatment, but it could also lead to greater access to CAR T-cell therapy for patients, as well as potentially reducing the cost of treatment. However, allogeneic cells can cause graft-versus-host disease (GVHD). GVHD occurs when the donated allogeneic cells are triggered by the patient's healthy cells and attack them. This can cause severe side effects, including death. Another serious reaction that can occur when allogeneic cells are introduced into the body is the opposite of GVHD, which is host-versus-graft. This is when the patient's immune system rejects the allogeneic CAR T-cells, which results in limited persistence.

Despite the limitations of allogeneic CAR T-cells, many researchers are investigating how this can be used to treat leukemia. A recent clinical study, presented at the American Society of Hematology Annual Meeting, generated allogeneic CAR T-cells derived from umbilical cords using CRISPR-Cas9 (Wang et al., 2025). To generate the cells, CRISPR was used to disrupt three key genes–TRBC, HLA-A, and HLA-B–and a lentivirus was used to insert CAR into the genome. These cells were then tested in a patient with relapsed acute lymphoblastic leukemia (ALL) that had a genetic mutation in the Tp53 gene, following chemotherapy. Preliminary results indicate that these allogeneic CAR T-cells are both safe and effective in treating patients with ALL. While further testing is needed, this data offers promise that allogeneic CAR T-cells can be used to treat patients, including those who have relapsed.

Another clinical trial is underway in the United States that tests CRISPR-generated allogeneic CAR T-cells for use in patients with relapsed or refractory acute myeloid leukemia (AML) (Caribou Biosciences, Inc., 2025). This study aims to use CAR T-cell therapy to target CLL-1, a cell-surface receptor that is highly expressed on AML cells throughout disease

progression, to test whether this method can successfully treat AML patients. The allogeneic CAR T-cells were created using Cas12a CRISPR technology, which utilizes a hybrid of RNA and DNA to guide the Cas enzyme for precise genomic editing, to make 5 genomic edits: (1) the *TRAC* locus was knocked out in order to eliminate the expression of the T-cell receptor, which reduces the risk of graft-versus-host disease; (2) anti-CLL-1 CAR was inserted into the TRAC locus to ensure uniform expression of CAR and to specifically target CLL-1; (3) PD-1 was knocked out in order to prevent T-cell exhaustion; (4) the *B2M* gene was knocked out in order to prevent T-cell rejection of these donor cells; and (5) the B2M–HLA-E-peptide was inserted into the *B2M* locus to reduce the possibility of rejection by natural killer cells (Daver et al., 2024). This method has proven successful in mouse xenograft models of AML, which showed reduced tumors and increased survival (Francica et al., 2024). The success of this mouse study has led to this Phase 1, multicenter clinical trial led by Caribou Biosciences, which is testing for safety and effectiveness in human patients, as well as determining dosing.

A research group in London is conducting a phase 1 clinical trial using CRISPR-edited allogeneic CAR T-cells to treat refractory B-cell acute lymphoblastic leukemia (B-ALL) (Ottaviano et al., 2022). They used traditional CRISPR-Cas9 to insert CAR into the TRAC locus of the T-cells. They also designed a virus targeted to the CAR T-cells that contains self-duplicating CRISPR-Cas9 machinery, which disrupts the CD52 gene in those infected cells and also in any copies of those cells. The removal of CD52 allows for increased survival of the CAR T-cells when combined with alemtuzumab, a treatment method for leukemia that specifically works by targeting the CD52 antigen. This CAR T-cell therapy was used to treat 6 kids with B-ALL and was combined with three other treatment options for leukemia: fludarabine (chemotherapy medication), cyclophosphamide (chemotherapy medication), and alemtuzumab (leukemia medication). 4 of the 6 patients infused with the engineered T cells showed cell expansion and achieved flow cytometric remission, which allowed them to proceed to allogeneic stem cell transplantation. Some manageable adverse effects that occurred during the trial were 2 patients who had grade II cytokine release syndrome that required biological intervention, one patient who developed transient grade IV neurotoxicity, and another who developed skin graft-versus-host disease that resolved after transplant conditioning. Overall, the side effects were within the expected range, and the study met its safety goals, which proves the feasibility of CRISPR-engineered immunotherapy in the future.

A separate Phase 1 clinical trial is utilizing CRISPR-based editing to create allogeneic CAR-T cells for use in patients with relapsed or refractory T-cell acute lymphoblastic leukemia (T-ALL) (Chiesa et al., 2025). Base editing is a precise gene editing technique that allows scientists to directly convert one DNA base pair into another. In this study, they used cytidine deamination to induce a C→T mutation in 3 genes–TCRαβ, CD52, and CD7–to create a triple genetic knockout in anti-CD7 CAR T-cells. These genes were knocked out to prevent graft-versus-host disease and to prevent the CAR T-cells from attacking each other. The

treatment was administered to 9 children and 2 adults. None of the patients had unacceptable adverse effects from the treatment, but some complications included cytokine release syndrome, rashes, multilineage cytopenia, and opportunistic infections. After 28 days, all patients had complete morphologic remission with no detectable leukemia cells observed under a microscope, and nine subjects (82%) achieved minimal residual disease remission as determined by polymerase chain reaction or flow cytometry testing. This remission allowed these patients to receive allogeneic hematopoietic stem cell transplantation, a treatment method that infuses healthy blood stem cells back into the patient's body. After this transplantation, 7 of the 11 patients (64%) were in ongoing remission at 3 to 36 months. Using these universal CAR T-cells on patients with relapsed or refractory T-ALL has shown promising preliminary results, leading to remission in most patients, which then allows for further treatment via allogeneic hematopoietic stem cell transplantation. While further testing is still needed in a larger group of patients, the success of this study offers promise for patients with difficult-to-treat or recurring leukemia.

Conclusion

Throughout history, leukemia has proven to be a devastating cancer that has taken many lives. It forms in the blood and bone marrow and affects many people in the United States and around the world. Even though there are several treatment options available, like chemotherapy, targeted therapies, and immunotherapy, it is still difficult to treat and leads to many deaths. Recent technological advancements, such as CRISPR gene editing technologies, have opened many possibilities for treating various cancers. It can be combined with CAR T-cell therapies as an alternative treatment option. These advancements in CAR T-cell therapies using CRISPR have shown lots of promise in treating leukemia. Numerous phase 1 clinical trials have proven successful in ensuring both the safety and effectiveness of CRISPR-edited CAR T-cell therapy for different types of leukemia.

Even though it has proven safe in phase 1 clinical trials, it has yet to move on to phase 2 clinical trials, which include a larger patient sample size, and this is because the use of CAR T-cell therapy for treating leukemia still has many limitations. Some prominent side effects that can occur include cytokine release syndrome, ICANS, and low white blood cell counts (Zhang et al., 2023). Even though these symptoms are usually not very serious and can be treated, they do limit the safety of the therapy and can lead to more severe consequences if not caught and managed early on. CAR T-cell therapy also comes with the risk of the host's immune system rejecting the allogeneic CAR T-cells and graft-versus-host disease. To mitigate this, CRISPR can be used in CAR T-cells to remove molecules that the host's immune system uses to recognize them or engineer the cells to evade an autoimmune response. Another major limitation is that, even though it is an advanced technology, CRISPR is not perfect and can

make off-target edits that cause unintended effects (Amiri et al., 2024). This can be solved by using alternative Cas nucleases that may increase efficiency and reduce off-target effects. Additionally, as CRISPR technologies continue to evolve, they are likely to become more precise and thus safer for use in humans. Despite the challenges that remain, CRISPR-engineered CAR T-cell therapy is advancing rapidly and holds a promising future for treating leukemia. Continued research could turn what is now an experimental therapy into a universal life-saving treatment for leukemia.

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