Implications of CRISPR in Immunotherapies for Leukemia

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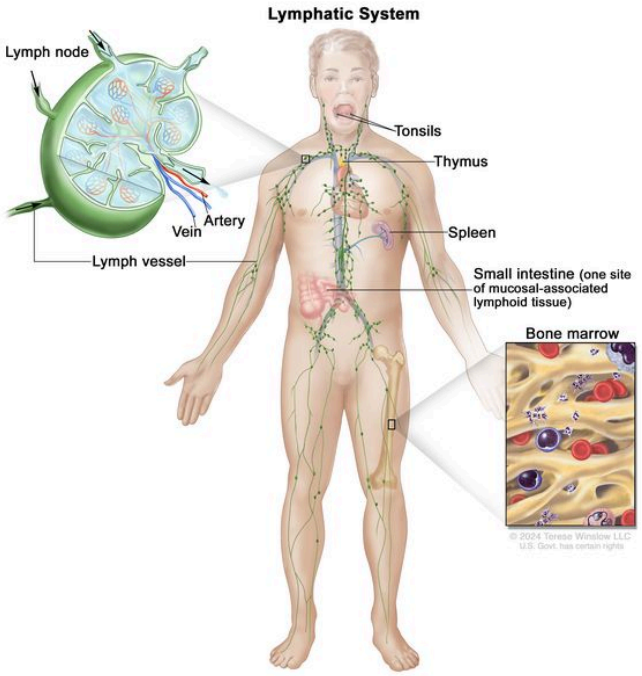
Abstract

Leukemia, a type of hematological (blood) malignancy, is an umbrella term used to describe a type of cancer in the blood cells and is one of the most common pediatric cancers in the United States. There are two broad categories of blood cells: red blood cells (RBC) and white blood cells (WBC). Red blood cells are formed in the bone marrow and are essential to transporting oxygen from the lungs through the body. White blood cells are crucial to fight infections and typically grow and divide in a regulated manner. However, those with leukemia produce an excessive amount of white blood cells or a limited amount of red blood cells, causing severe harm to the body. Traditionally, leukemia is commonly treated with chemotherapy, cell transplantation, and radiation. Yet, these methods offer limited success rates and often diminish patients' quality of life. As leukemia arises as a significant contributor to adolescent mortality, alternative approaches are needed to treat it and improve survival rates. One such approach is combining immunotherapies, treatments that utilize the immune system, and CRISPR, a genome editing tool, thus enabling the body to use the immune system to fight leukemia instead of the traditional treatments such as chemotherapy and radiation. The review discusses the immune system's role in leukemia progression and how immunotherapies, augmented by CRISPR, offer targeted and effective alternatives to traditional therapies. Recent studies showcasing CRISPR-engineered T cells with chimeric antigen receptors (CARs) targeting leukemia cells are highlighted for their promising outcomes, including improved precision and prolonged efficacy compared to conventional treatments.

Introduction

Leukemia is the most common cancer in children under 15 years in the U.S. However, 70% of cases occur in those aged 55 to 84 years (National Cancer Institute, 2015). Each year, there are around 60,000 new cases of leukemia globally, resulting in approximately 23,000 deaths, with a survival rate of 66.7%. When diagnosed with leukemia, the total cost for treatment can range anywhere from $340,000 to $700,000 (American Cancer Society, 2024). Leukemia emerges in the blood-forming tissues, such as the bone marrow and lymphatic system, generally causing an abnormal increase in mutated white blood cells, which are crucial for fighting infections, leading their functions to be hindered.

Figure 1
Lymphatic System and Bone Marrow



From: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/lymphatic-system

The propagation of these cancerous cells starts when a bone marrow stem cell's DNA acquires a mutation during replication (cell division), causing the new daughter cells to grow excessively and divide in a dysregulated manner. As the population of these mutated cells increases, they overtake the healthy cells and inhibit the body's ability to function properly (MD Anderson Cancer Center). Due to the critical role of white blood cells in the body's immune system, untreated leukemia can result in an inability to fight against viruses, bacteria, and other invading substances (University of Rochester Medical Center). This leads to a high rate of recurrent infections, weakness, and/or anemia in patients with leukemia (Cancer Council Australia). However, due to the heterogeneity of leukemia, symptoms can significantly vary between those affected.

Leukemia Overview
Leukemia can be classified into two categories; how fast the leukemia progresses - chronic or acute and the type of white blood cell affected - lymphoid or myeloid. Acute leukemia refers to the rapid growth and division of the cancerous cells. However, chronic leukemia indicates that the cells multiply over long periods, causing their symptoms to arise slowly. Chronic leukemia usually occurs in mature blood cells. Lymphocytic leukemia impedes the ability of lymphoid cells (lymphocytes) to form lymphatic tissue that builds the immune system. On the other hand,

myelogenous leukemia interferes with the myeloid cells, which create red, white, and platelet-creating cells.

There are four main types of leukemia (Mayo Clinic Staff, 2022):

- Acute lymphoblastic leukemia
- Acute myelogenous leukemia
- Chronic lymphoblastic leukemia
- Chronic myelogenous leukemia

Acute lymphoblastic leukemia (ALL) is the most prominent type of cancer in children. ALL progresses rapidly and replicates immature lymphocytes, which cannot fulfill their role, harming the immune system. (National Cancer Institute, 2015)

Acute myelogenous leukemia (AML) is the most prevalent type of leukemia in adults, while also occurring in children as well. AML begins in the myeloid cells of the blood marrow and may rapidly spread to other parts of the body. (American Cancer Society, 2018

Those with chronic lymphoblastic leukemia (CLL) produce an excessive amount of lymphocytes, a diagnosis that slowly deteriorates one's body. It is the most frequent form of leukemia in adults and is very rarely seen in children (National Cancer Institute, 2015).

Chronic myelogenous leukemia (CML) is the least severe subtype of leukemia, but it is also the more rare, especially in children. In CML, myeloid cells are produced in surplus. Those living with CML have a bright prognosis, and most reach remission (Mayo Clinic Staff, 2023).

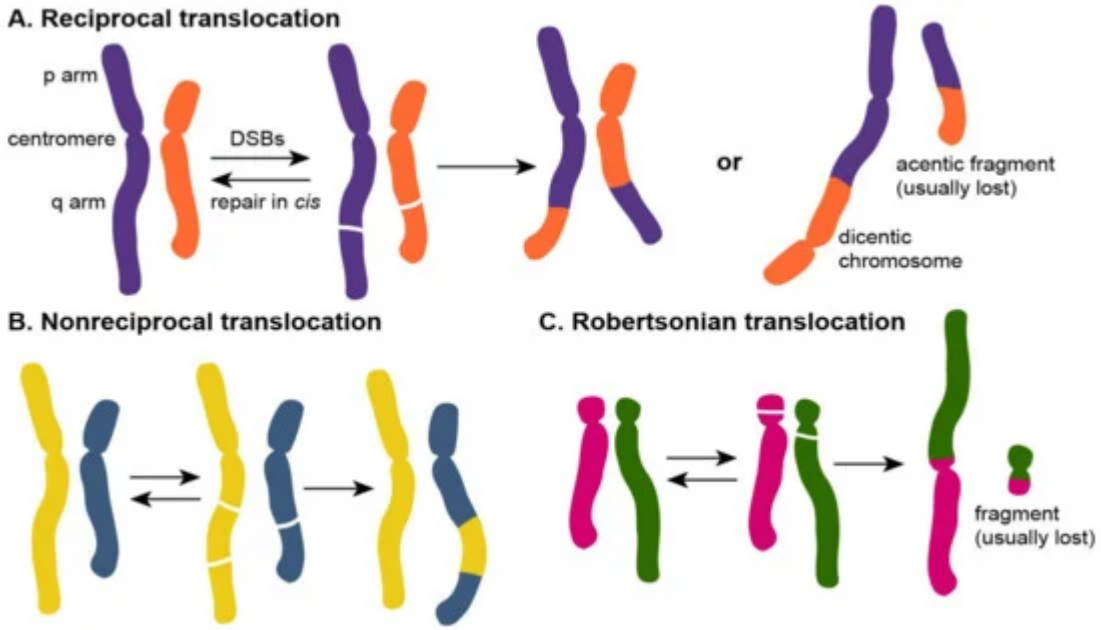
Risk Factors of Leukemia

The causes of leukemia are not well elucidated, particularly acute leukemia. While genetic factors may be at play, several environmental factors put individuals at a higher risk. These include exposure to intense radiation or certain chemicals (i.e., benzene), smoking, infection by specific oncogenic viruses, like the Human T-Cell leukemia virus (HTLV) and the human papillomavirus (HPV), or previous cancer treatments (Mayo Clinic Staff, 2022). All of these environmental exposures can lead to mutations within an individual's DNA, thus leading to an increased chance of leukemia

Additionally, a family history of leukemia or specific genetic disorders, such as Down syndrome or Klinefelter syndrome, also increases the chances of developing leukemia (Cleveland Clinic, 2022). While not the sole cause, chromosomal translocations, where DNA from one chromosome moves to another chromosome, can also result in leukemia (MedlinePlus). This genetic change is called the Philadelphia chromosome, and it is a common cause of CML

(Cancer Council Australia). Excessive radiation exposure can induce these genetic changes and thus is a high-risk factor for developing leukemia (Tachibana et al., 2022).

Figure 1
Chromosomal Translocation Types



From: https://www.mdpi.com/2072-6694/14/20/5110

Symptoms
Patients with leukemia may suffer from complications due to these cancerous cells interfering with the production of normal white blood cells. Patients report bone pain, most commonly felt in the legs, arms, ribs, and sternum, as cancerous cells overtake the bone marrow (Zajączkowska, 2019). This disease may also cause harm to the intestinal tissues. Leukemia creates lesions affecting the stomach, ileum, or proximal colon, leading to inflammation, bleeding, or infections (Tagliaferri, 2023). If cancerous cells infect the blood vessels, patients develop potentially fatal issues, such as ischemic cardiac disease (coronary artery disease) in adults, where the heart does not receive the required amounts of blood and oxygen. Most importantly, leukemia targets the immune system, making those suffering vulnerable to developing severe infections and prolonged recovery (Sherrell, 2023).

Standard Treatments
Current treatment options for leukemia vary by type but typically involve chemo- or radiation therapy, blood stem cell or bone marrow transplantation, or surgical removal of the spleen (Cancer Council Australia). Chemotherapy is the most common form of treatment for leukemia patients, and it involves the use of chemicals to kill or stop the cell division of leukemia cells (Cleveland Clinic, 2022). Cell transplantation is another standard therapeutic method as it

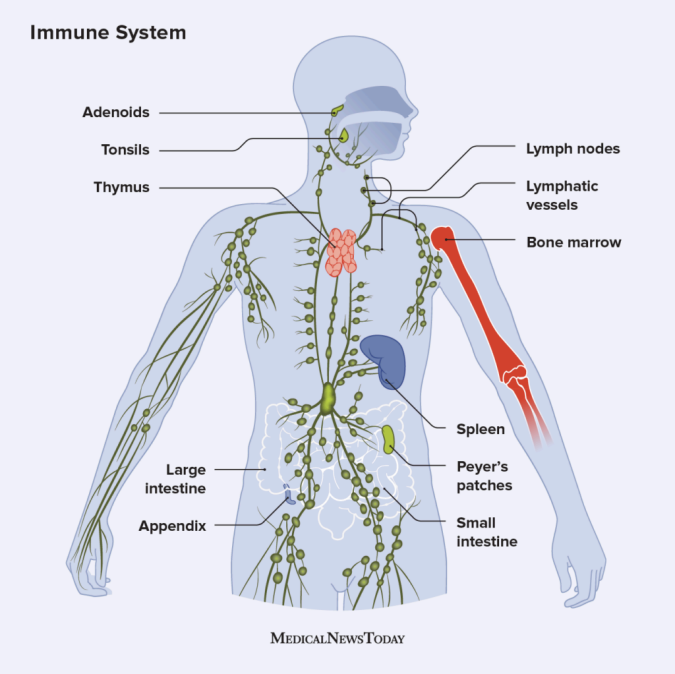
focuses on removing cancerous cells through irradiation and replacement with new, healthy hematopoietic cells from a donor (Leukemia & Lymphoma Society, n.d.). Despite the various therapeutic options, the 5-year survival rate varies from 31.7% to 88.0% (SEER, 2023). Over the years, immunotherapies have served as a promising therapeutic that has helped increase the survival rate for leukemia (Huang, Cancer Research Institute).

The Immune System

Leukemia weakens the immune system by disrupting the production and function of white blood cells, which are integral to the immune response. The immune system is an intricate network comprising a multitude of cells, tissues, and organs working together to defend the body against an array of harmful substances, including pathogens, abnormal cells, and other foreign invaders.

Figure 2

The Immune System



From: https://www.medicalnewstoday.com/articles/320101

Critical components of the immune system include the white blood cells, also known as leukocytes, playing a vital role in defending the body against infections and foreign invaders. The lymphatic system - consisting of the lymph nodes, spleen, thymus, and lymphatic vessels, helps circulate and filter lymph (a fluid containing white blood cells) throughout the body, facilitating immune responses (Better Health Channel, 2022; Canadian Cancer Society). The bone marrow, soft, spongy tissue located within the bone cavity, holds the hematopoietic stem cells that are responsible for differentiating into blood cells, including erythrocytes (RBCs),

leukocytes (WBCs), and platelets, essential to clotting. The hematopoietic stem cells separate into myeloid stem cells or lymphoid stem cells. In a healthy immune system, the lymphoid stem cell becomes a lymphoblast cell and further separates one of three types of lymphocytes: B cells, T cells, and natural killers. In a healthy immune system, stem cells regenerate in response to deficiencies to restore balance (Cancer.Net). However, in an immune system impacted by leukemia, immature and abnormal white blood cells are excessively produced from the bone marrow, impeding the production of red blood cells, and causing anemia and thrombocytopenia resulting from a lack of platelets. The immune system becomes severely compromised due to a deficiency in red blood cells, platelets, and healthy white blood cells, impairing its ability to defend the body against foreign substances effectively. This compromised state contributes to the increasing rates of cancer cells, which can go undetected.

Leukemia suppresses the immune system, leading patients to face various complications with their ability to respond to immune insults (Murphy, 1993). Leukemia is most common in lymphocytic white blood cells, either B or T cells (Cancer.Net, 2017). B cells protect our body by initiating targeted attacks on foreign invaders, like viruses and bacteria, through our bloodstream. T cells are responsible for the body's cells that can no longer perform adequately, most often caused by infections or diseases like cancer. As they grow and mature, B cells can generate immunoglobulins and other antibodies, which play a vital role in the immune system by fighting off infections and improving overall health. However, the B cells of one with leukemia never reach the point of full maturity and instead rapidly begin to divide (Murphy, 1993). As a result, B cells cannot produce functioning antibodies to fight off infections, compromising the patient's immune system and leaving them more vulnerable to illnesses. T cells also play a role in immunosuppression, as their receptors can recognize and bind to molecules that inhibit immune responses, thus hindering the immune system's abilities (Cleveland Clinic, 2023).

Immunotherapies

CLL cells manipulate T lymphocytes to gain a survival advantage by turning off cytotoxic T cell function, likely due to defective linker for activation (LAT) of T cells (Pincus, 2022). Another way in which CLL affects the immune system is by inhibiting the proper functioning of T cells, which are responsible for our autoimmune response. Patients with CLL are known to develop autoimmune diseases, such as autoimmune cytopenia (destruction of hematologic cells), in which their immune system begins to attack red blood cells and platelets. However, treatments are available to help control these flare-ups.

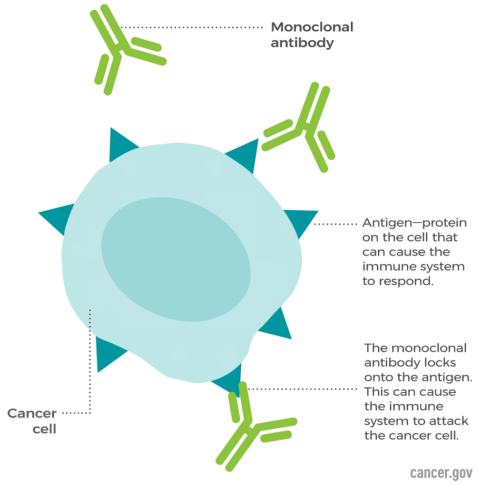
Types of Immunotherapies used for leukemia treatment:

- Monoclonal antibodies
- T-cell transfer therapy

- Immune checkpoint inhibitors

Antibodies or immunoglobulins are proteins circulating through the body until they find their corresponding antigen, allowing them to destroy cells containing the antigen (American Cancer Society). Antibodies are shaped like a Y; the tip comprises a specific paratope that binds with the matching epitope on the antigen. Monoclonal antibodies are synthetically produced to acquire a paratope that can bind with cancer cells, disabling their function. After production, they are intravenously injected (IV) (LibreTexts Medicine).

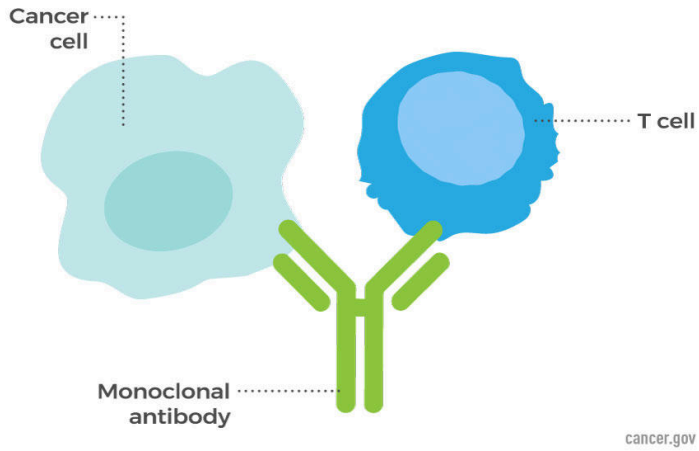
Figure 5
Monoclonal antibody marking a cancer cell



From:
https://cancer.gov/sites/g/files/xnrzdm211/files/styles/cgov_article/public/cgov_image/media_image/2019-12/Monoclonal-antibodies-illustration.gif?itok=INBgYvqT

Rituximab, a type of monoclonal antibody, binds to a CD20 protein expressed on the cell surface of normal and cancerous B-cells, flagging them so the immune systems can kill them. Some antibodies are bispecific (bind to many proteins), like Blinatumomab, which binds to both CD19 on leukemia cells and CD3 on T-cells from the immune system, allowing T-cells to get into a closer proximity with leukemia cells and attack them. (LibreTexts Medicine)

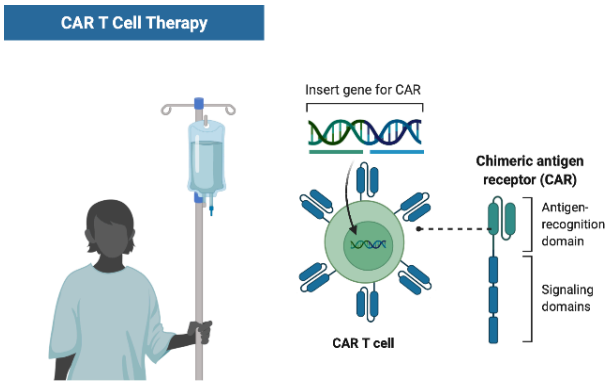
Figure 4
Bispecific Y-shaped antibody binding to a T-cell and Cancer cell



From:
https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/monoclonal-antibodies

CAR T-cell therapy harnesses the power of the immune system to target and destroy cancer cells. T-cells have unique receptors that recognize antigens, enabling the identification of foreign antigens in the body and signaling an immune response. Yet, cancer cells aren't often recognized by these regulatory cells and continue to multiply (Penn Medicine). Chimeric antigen receptor (CAR) T cell therapy modifies the body's CD8 T-cells so they do not overlook cancer cells and can attack them. A patient's T-cells are first extracted and genetically modified in a laboratory to express CAR, creating a CAR T-cell. Millions of T-cells are altered and infused back into the body through an IV, allowing them to bind to the antigen proteins of cancer cells such as CD19 and BCMA and kill them. (MD Anderson Cancer Center, National Cancer Institute, 2022)

Figure 3
CAR T-cell Therapy



Obtained from: https://www.biorender.com/template/car-t-cell-therapy

The immune system has built-in checkpoints that regulate its activity and prevent excessive responses. However, cancer cells can exploit these checkpoints to evade detection by the immune system. Immune checkpoint inhibitors block these checkpoints, preventing T-cells from being released in large quantities and allowing more t-cells to recognize and attack cancerous cells (National Cancer Institute). Key immune checkpoints include programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). PD-1 is blocked by anti-PD-1, and CTLA-4 is blocked by anti-CTLA-4 antibodies, terminating the progression of cancer proliferation.

CRISPR-based Leukemia Therapeutics

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) sequences were first identified in 1987 as unusual repeated DNA sequences in Escherichia coli (E. coli) bacteria genomes. In 2007, it was discovered that prokaryotes used these regions in the immune system. The immune system utilizes CRISPR to record the phages that once invaded the system and eliminate the phages upon future invasion. 2012 marked the discovery that the CRISPR-Cas system could be harnessed as a powerful genome editing tool (Addgene). The CRISPR-Cas 9 technology can edit parts of the genome through the removal, addition, or alteration of sections of the DNA sequence. The system acquires a guide RNA (gRNA), a synthesized strand of RNA designed to be a complementary match with the target gene's DNA sequence requiring alteration. gRNA is embedded within an RNA scaffold and attaches to the DNA. The Cas9 enzyme, which behaves like a pair of scissors cutting the two strands of DNA, is guided by the gRNA sequence to cut and alter the accurate location of the genome (Your Genome). CRISPR technology has been gaining significant attention recently and is being explored in various fields.

CRISPR technology has emerged as a groundbreaking tool in immunotherapies, offering innovative methods for treating cancer and disease. One promising application involves the use of CRISPR to enhance the body's immune system to eliminate cancer cells. CRISPR can be used to precisely edit the genome of immune cells, such as T cells, to target and destroy cancer cells specifically. (Beckman Coulter Life Sciences). For example, CRISPR can be used to engineer immune cells to express chimeric antigen receptors (CAR), which provides the ability to recognize and attack cancer cells. This method, called CAR-T cell therapy, is a powerful form of cancer treatment. CRISPR enhances this treatment since it can more accurately edit the genes of T cells. These advancements in CRISPR-based immunotherapeutics hold significant potential for personalized medicine, paving the way for more precise, efficient, and targeted treatments, which can be tailored to individual patients based on their unique genetic makeup.

CRISPR Immunotherapies for Leukemia

CRISPR technology has demonstrated remarkable success in immunotherapies for leukemia, offering a highly targeted and personalized approach to treating this blood cancer. In particular, researchers have focused on engineering T cells using CRISPR to express CARs targeting surface proteins found on leukemia cells. In a recent study, researchers used CRISPR to knock out specific genes while adding DNA to potentially make T cells more robust and persistent (Stadtmauer et al., 2020). Through precise genetic editing facilitated by CRISPR, these engineered T cells become highly potent in selectively attacking and destroying leukemia cells while sparing healthy cells. Despite encountering off-target effects, the CRISPR-edited cells persisted for at least 9 months, showcasing promise for improved longevity compared to traditional CAR-T cell therapy. The study represents a significant step in utilizing CRISPR for leukemia treatment. However, outcomes were modest, and further experimentation and optimization are needed to enhance the efficacy of this innovative approach (Jennifer Couzin-Frankel, 2020).

CRISPR-Cas9 has also been used to edit the genome of CLL cells. Chronic Lymphocytic Leukemia is a heterogenous B-cell neoplasia created by over 60 recurrently mutated genes, but their specific contributions to the disease remain unclear. Genetically engineered mouse models and human cell lines have been used to decipher the mutations' significance. Still, they cannot accurately depict CLL cells due to many discrepancies with the primary cells. This is where CRISPR-Cas 9 genome technology comes into play; researchers use primary human CLL cells for ex-vivo genetic manipulation (Mateos-Jaimez et al., 2022). Stimulatory signals from the tumor microenvironment are crucial for CLL cells, posing a challenge for gene transfer methods. The solution creating a cell culture system that mimics a lymph node environment, enabling the retroviral gene transfer. The first gene target was the CD19 gene, a pan B-cell marker in CLL cells. The Cas9-CD19 sgRNA ribonucleoprotein complex was introduced to the cryopreserved cells, and 72 hours after electroporation, over 85% of cells had been downregulated with Cas9-ATTO550 sgRNA ribonucleoprotein. Following this success, they target the cell cycle regulatory protein Cyclin D2 (CCND2), upregulated in CLL cells. The CCND2 knock-down (KD) efficiency was 70% and 60%. CCND2 KD inhibited cellular proliferation, resulting in a substantial decrease in cell growth ranging from 50% to 70% when utilizing two sgRNAs, compared to non-targeting controls, within a mere 48-hour timeframe. This study overcomes limitations associated with cell lines and mouse models and offers a more representative model for investigating the complexities of CLL pathogenesis.

CRISPR has allowed researchers to identify novel genes associated with blood cancers, one prominent example being Staufen 2 (Stau2), a regulator for the growth and creation of leukemia stem cells, the cells responsible for therapy resistance and cultivating both CML and AML (Bajaj

et al., 2020). Before the finding of Stau2, they originally discovered a broad group of RNA-binding proteins (RBPs) in which six of the RBPs showed prominent upregulation in leukemic stem cells. This study's application of CRISPR technology laid the foundation for novel immunotherapeutic strategies for leukemia (University of Rochester Medical Center). Identifying cellular signals critical to cancerous growth, especially in aggressive forms of myeloid leukemias, sets the stage for targeting these signals with genome editing interventions. Manipulating genes like Stau2 to block cancer proliferation opens up an array of possibilities in designing precise and targeted immunotherapies for each individual battling with blood cancer.

In the broader context of leukemia immunotherapies, using CRISPR technology opens avenues for further targeted interventions. Even though these methods have only been utilized in smaller studies, the potential of this technology is to be able to edit cells from the body directly, either ex-vivo or in-vivo. This approach presents an opportunity to create healthy cells resistant to cancerous growth, offering a potential therapeutic breakthrough for leukemia treatment.

Ethics of CRISPR

While CRISPR opens a whole new realm of possibilities regarding leukemia, and all genetic diseases for that matter, the ability to edit the human genome raises questions about this tool's potential misuse and unintended consequences. Researchers and bioethicists argue that though it may begin solely for therapeutic purposes, it may quickly be manipulated for enhancement purposes (National Human Genome Research Institute, 2017). CRISPR technology is relatively new; thus, the prospective consequences haven't been explored yet, making safety a primary concern. There are many potential, unpredictable side effects, such as off-target effects or edits in the wrong place and mosaicism, where only some cells receive the needed edits, creating discrepancy. The future of CRISPR remains far away as researchers who attended the International Summit on Human Gene Editing ultimately agree that until thorough testing has been done and deemed safe, CRISPR cannot be used by the public for clinical purposes, as the possible risks outweigh the positives (National Human Genome Research Institute, 2017). Furthermore, the cost of these cutting-edge treatments adds up to a hefty sum. When considering the specific immunotherapies and stem cells catered toward each patient, along with customized genome editing, the total cost is most likely far out of reach of the general public, even with insurance, pushing the accessibility of treatment further away.

Conclusion

Leukemia is the second leading cause of childhood mortality by cancer in the United States, with over 300,000 children worldwide diagnosed annually, but an effective cure has yet to be developed (Leukemia and Lymphoma Society, 2023). Current traditional treatments, including chemotherapy, radiation, and bone marrow transplantation, only generate a 65.7% survival rate while also diminishing patients' quality of life (Kandola, 2024). Immunotherapies, alongside

CRISPR technologies, pave a new pathway for targeted treatments, opening up promising possibilities for a cure. By utilizing the body's immune system to target and rid cancerous cells, existing treatments can be redefined as a complement to chemotherapy and potentially a standard course of treatment for tackling leukemia.

CRISPR technology has shown significant success in leukemia immunotherapies, enabling precise genetic modifications in T cells to express CARs targeting leukemia cells. Compared to traditional, first-generation CAR-T cell therapies, CRISPR-edited T cells remained effective for 9 months, showing more tremendous success (Stadtmauer et al., 2020). Furthermore, CRISPR-Cas9 has created a more accurate representation of CLL pathogenesis by overcoming the constraints of traditional research models. It can edit the genome of CLL cells through *ex-vivo* genetic manipulation. Researchers have successfully targeted genes like CD19 and Cyclin D2, allowing for the downregulation and inhibition of cellular proliferation (Mateos-Jaimez et al., 2022). Subsequently, CRISPR technology has enabled the discovery of novel genes essential to leukemia, such as Staufen 2 (Stau2), which plays a crucial role in the growth and development of leukemia stem cells (Bajaj et al., 2020). This breakthrough, facilitated by CRISPR, lays the groundwork for innovative immunotherapeutic approaches targeting crucial cellular signals implicated in cancer progression. As research in immunotherapies and CRISPR technology advances, ongoing clinical trials and collaborative efforts will be essential to optimize treatment outcomes and address the remaining ethical concerns of genome editing. Future research aims to determine the long-term durability of CRISPR-edited CAR-Ts in the body. Together, this new wave of treatment marks a significant step forward in the fight against leukemia, offering hope for patients through the possibility of a cure for this devastating disease.

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