

Treating Leukemia Using CRISPR-Cas Genome Editing to Improve Delivery of Allogeneic and Autologous Stem Cells

Rishabh A. Shah

Westlake High School, 4100 Westbank Dr, Austin, TX, 78746, USA; rishi.329.shah@gmail.com

Mentor: Dr. Hamidreza Shaye

ABSTRACT: Every 27 seconds, someone across the world is diagnosed with blood cancer. The global burden of leukemias and other blood cancers has increased despite interventional therapies such as chemotherapy, immunotherapy, and adult stem cell transplantation. This paper explores the use of precision biotechnologies such as the CRISPR-Cas gene-editing tool to improve patients' quality of life, refining the delivery method of regenerative blood stem cell therapies in leukemias. In allogeneic blood stem cell transplantation, CRISPR-Cas can facilitate immune tolerance to donor hematopoietic stem cells by cleaving parts of their Human Leukocyte Antigen (HLA) genes and by adding genes encoding protective proteins. This gene-editing technique thereby aids in transforming the limited blood stem cell donor pool to create a safer and more effective interventional therapy without requiring the close matching of HLA between the donor and the recipient. In contrast, it also functions to edit the patient's own blood stem cells in blood cell disorders like sickle cell anemias and thalassemias in order to provide long-lasting, disease-free survival. Current advances in genetic engineering technologies are poised to revolutionize the future of regenerative biomedicine, mobilizing the universal delivery of off-the-shelf stem cell products.

KEYWORDS: Biology, Oncogenetics, CRISPR-Cas, Leukemia, Stem Cell Transplantation (SCT).

■ Introduction

Global Burden of Leukemias:

Globally, leukemia is the second most common blood-related malignancy across all ages as of 2022.¹ Leukemia is the most common cancer among children less than 5 years old and has the highest mortality rate in early childhood.² In 2020, leukemias were the 11th most frequent cause of cancer-related mortality worldwide, causing more than 300,000 deaths.²

Defining Leukemia and the Subtypes of Leukemias:

Leukemia is a life-threatening blood cancer caused by the abnormal and uncontrolled proliferation of immature white blood cells. It is a cancer related to hematopoiesis, the production of blood cells, and exhibits diverse genetic characteristics.³ Leukemias can be classified into subtypes based on the progenitor cell type, the speed of abnormal cell growth, and the stage of maturation arrest.³ The major subtypes include: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML).⁴ Table 1 displays the characteristics of the four major subtypes of leukemias and provides additional information regarding them.⁶⁻¹⁵ Of these subtypes, AML is the most common acute leukemia among adults and has the highest adult mortality rate among all leukemias. AML has an overall five-year survival rate of 32% for adult patients over 20 years and a five-year survival rate of 18% for elderly patients over 65 years, depicting the threat leukemia poses worldwide.⁵ At the same time, ALL, the most common leukemia among children and adolescents, accounts for the highest mortality rate, 14%, in that age range.²

Table 1: Subtypes of leukemias with associated mutations, the most affected population, and the percentage proportion of all major leukemia subtypes; created in BioRender. A chunk of rarer leukemias, such as hairy cell leukemia and large granular lymphocytic leukemia, are omitted; hence, the ranges in the "Percentage of leukemias" column do not add up to 100%. Breakpoint Cluster Region - Abelson murine leukemia viral oncogene homolog 1 fusion gene (BCR-ABL1). Philadelphia chromosome (Ph+). Fms-like tyrosine kinase 3 (FLT3). Nucleophosmin 1 (NPM1). Tumor Protein P53 (TP53). Short arm ("p") of chromosome 17 (17p). Data were acquired from a variety of sources.⁶⁻¹⁵

Subtypes of Leukemias

Type	Mutations associated	Primary population	Percentage of all leukemias	References
ALL	BCR-ABL1 (Ph+)	Pediatric	10-12%	[6 - 15]
AML	FLT3, NPM1, BCR-ABL1 (Ph+)	Adults	31-35%	[6 - 15]
CLL	TP53, 17p	Adults	33-38%	[6 - 15]
CML	BCR-ABL1 gene (Ph+)	Adults	15%	[6 - 15]

Etiology and Pathophysiology of Leukemia:

Often, leukemias can be caused by a variety of factors, including genetic mutations and environmental factors.⁴ Genetic mutations can be either inherited via syndromes such as Down syndrome or Li Fraumeni syndrome, or acquired from unsafe exposure to radiation or chemicals. An example of this is the BCR-ABL fusion gene, which arises from an acquired mutation from a chromosomal translocation identified as the Philadelphia chromosome (Ph), ultimately leading to the development of CML and AML. Environmental factors that

may cause leukemia include ionizing radiation from radiation therapy or nuclear accidents, exposure to cigarette smoke, exposure to chemicals such as benzene and formaldehyde, and viruses such as Human T-Lymphotropic Virus-1 (HTLV-1) and Epstein-Barr virus (EBV).¹⁶

Leukemic stem cells (LSCs) are abnormally mutated hematopoietic stem cells in the bone marrow. LSCs are a critical component of leukemia because they trigger the onset of AML, causing uncontrolled cancer cell growth and thereby increasing their intrinsic resistance to chemotherapy.¹⁷ The malignant transformation of hematopoietic progenitor cells (HPCs) can lead to an abnormal accumulation of immature cells in the bone marrow. This process causes an overflow of white blood cells and interferes with regular blood cell production.¹⁶ In addition, Interleukin-1 (IL-1), a group of cytokines (signaling proteins), is overproduced in AML, while normally they are underproduced. Interleukin-1 receptor accessory protein (IL-1RAP) overproduction has been traced to myeloid malignancies in its signaling pathway, and it is involved in processes such as rapid cancer cell proliferation and leukemic cell shielding, which can further promote the progression of AML.¹⁸ Hence, to limit the progression, it is imperative to target IL-1RAP to rupture those downstream pathways that would otherwise lead to cancerous transformation.¹⁸

With these multiple mechanisms of complex interactions triggering mutations and the proliferation of immature white blood cells, the body's primary immune defense becomes compromised and dysfunctional. Along with it, overcrowding in the bone marrow, as seen in Figure 1, can lead to the suppressed production of other cell lines like red blood cells and platelets, leading to anemia and clotting interference, respectively.^{3,19} These pathological processes may result in symptoms such as fever, fatigue, pallor, bone pain, bleeding and easy bruising, recurrent infections, and lymph gland enlargement, among many others, as seen in Figure 2.²⁰

The Effects of Leukemia on the Human Body: Pathophysiology and Symptoms of Leukemia

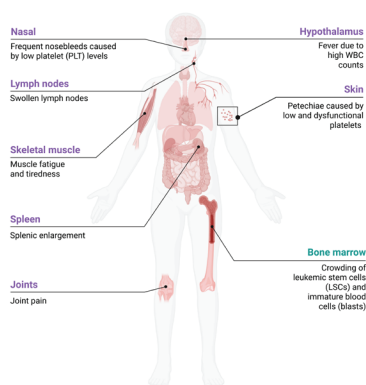


Figure 1: A portrayal of the “crowding out” aspect of leukemia in which a rapid increase in the amount of blasts and leukemic stem cells (LSCs) in the bone marrow can lead to implications for all three blood cell lines as listed above. This figure was made in BioRender and adapted from an image by Hamid, 2013.¹⁹

The Crowding of Blasts and Leukemic Stem Cells (LSCs)

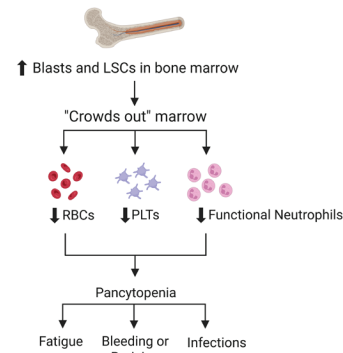


Figure 2: A visual depiction of the pathophysiology and common symptoms of leukemia. This figure was made in BioRender, and the information was gathered from Khwaja *et al.*, 2016.²⁰

Introduction to Leukemia Therapies:

Current leukemia treatments include standard interventional therapies such as chemotherapy, immunotherapy, and radiation therapy, as well as advanced therapies like CAR-T cell therapy and stem cell transplantation.²¹ These therapies are discussed in more depth later in this paper, focusing both on their clinical effectiveness and the adverse events that can impact patients' quality of life. Stem cell transplantation, the Human Leukocyte Antigen (HLA), and CRISPR-Cas9 genetic engineering in stem cell transplantation are introduced below.

Stem Cell Transplantation: Advanced Therapeutic Potential in Leukemias:

Stem cells are a subtype of self-renewing cells in our body tissues, with the ability to differentiate into specific cell lines: hematopoietic (blood-producing), mesenchymal (resembling embryonic connective tissue) cells, and others.¹⁷ For this review, we will restrict our discussion to hematopoietic stem cells (HSCs), which can regenerate new healthy blood cells and replace the existing diseased cells. This ability of HSCs makes them an effective therapeutic option in leukemias and blood cell disorders such as hemoglobinopathies.

Allogeneic (allo-SCT) and Autologous (auto-SCT) Stem Cell Transplantation:

Stem cell transplantation is a cellular therapy that can potentially restore damaged tissue through its regenerative properties. There are two subtypes of stem cell transplantation—autologous (auto-SCT), consisting of the individual's own stem cells, and allogeneic (allo-SCT), where stem cells from a matched donor are obtained. These matched donors may be a family member or unrelated to the patient. While auto-SCT is more accessible and timely, it does pose a risk of relapsed leukemia due to possible residual disease, thereby limiting progression-free survival.²² Allo-SCT is notably more effective in treating leukemia in contrast to auto-SCT.^{22,23} However, it requires close matching of the Human Leukocyte Antigen (HLA), an immune-recognition marker of self versus foreign, between the patient and the donor. HLA compatibility helps prevent the rejection of transplanted tissue as well as re-

duces the probability of an adverse immune response mounted by the transplanted tissue against the patient's normal tissues, known as graft-versus-host disease (GVHD).²⁴ Despite these benefits, it is critical to recognize that the process of securing an HLA-matched donor can be time-consuming and burdensome.

Human Leukocyte Antigen, Immune Tolerance, and Allo-SCT:

HLA proteins are surface markers on leukocytes (white blood cells) and are encoded by Major Histocompatibility Complex (MHC) molecules, which are critical components of our immune system. They are Class I and Class II genes located on the short arm of chromosome 6.²⁵ There are three loci for Class I (HLA-A, HLA-B, and HLA-C); each individual inherits A, B, and C from each parent, resulting in six allelic pairs in each cell.²⁵ Whereas Class II molecules are mainly expressed on immune cells, especially those able to present foreign proteins (antigens). They have three loci (HLA-DR, HLA-DQ, and HLA-DP), of which HLA-DR is the most prominent and polymorphic subtype. HLA proteins are responsible for initiating immune rejection of foreign pathogens from their position on the surface of cells, because of their ability to differentiate self from non-self.²⁶ It is imperative to note that HLAs align more closely with other HLAs of the same family and next with those of the same ethnicity, due to the unique set of genes that comprise them. Each ethnicity presents its own unique HLA profile created by varying frequencies of inherited alleles.²⁷ Allo-SCT requires close HLA matching (the process of identifying and matching all HLA pairs) to prevent rejection of the transplant and GVHD. The need for HLA typing and matching is currently a huge barrier to access to stem cell therapy in leukemia.

Gene Editing Biotechnologies: TALENs, ZFNs, and CRISPR-Cas:

The focus of this review is on the utilization of scientific advancements in precision medicine biotechnologies like transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats - Cas endonuclease) for the timely and efficient delivery of stem cell therapies.²⁸ The revolutionary potential of such gene-editing techniques, which are evolving to fix gene mutations and add functional genes *ex vivo*, has redefined both transplantation and regenerative.²⁹

CRISPR-Cas: the Present and the Future:

Genetic editing biotechnologies, especially the more precise CRISPR-Cas system, have shown promise to make allo-SCT more universal, precluding the need to closely match the HLA of the donor with that of the patients. This gene modification tool uses the HLA-abrogation process, consisting of the disruption and self-repair of stem cell DNA.³⁰ This precision delivery technique would allow allo-SCT regimens with superior effectiveness to induce prolonged remission of leukemias while also being available relatively quickly. Additionally, this

technology will help in minimizing the likelihood of adverse events such as GVHD.³¹ Moreover, incorporating CRISPR-Cas genome editing to repeal HLA-mediated immune rejection will limit the need for immunosuppressive drugs with serious side effects.³² CRISPR-Cas gene editing has also shown great success as a therapeutic approach in the improved delivery of autologous stem cells with the utilization of *ex vivo* gene editing to “silence” or “reactivate” certain responsible target genes.³³ This aspect of CRISPR-Cas gene editing has been elaborated in the discussion section of this paper.

Gene Editing, Stem Cell Transplantation, and Hemoglobinopathies:

Genetically inherited red blood cell disorders such as sickle cell disease and beta-thalassemia major are the most common inherited diseases around the world, affecting 7% of the world's population.³⁴ Although non-cancerous, their global fatality at a staggering 376,000 lives lost annually positions them as a major global health issue.³⁴ The overall disease burden of these conditions makes it critical to work on prevention and therapies. CRISPR-Cas9 gene-editing technology has promise in enabling long-lasting disease-free survival for patients with these blood cell disorders through edited autologous stem cell transplantation.

■ Discussion

Current Therapies in Leukemia:

Current interventional therapies for blood cancers include chemotherapy, radiation therapy, targeted therapies such as tyrosine-kinase inhibitors, novel immunotherapies such as immune checkpoint inhibitors, natural killer-mediated immunotherapy, and chimeric antigen receptor (CAR) T-cell therapy, and monoclonal antibodies. These therapies have indications, advantages, and limitations, such as adverse effects that may cause poor quality of life or failure of treatment itself. Hence, the usage of stem cell transplantation as a therapeutic approach for blood cancers becomes essential in refractory and relapsed leukemias. The characteristics of and statistics related to these therapies are displayed in Table 2.⁴⁷⁻⁵⁰

Chemotherapy:

Chemotherapy is the most widely used standard of care treatment protocol for leukemias.³ Chemotherapy is a therapeutic cancer intervention that causes genotoxicity to damage cellular DNA.²¹ The chemical agents produce reactive oxygen species—unstable and reactive molecules, which have the potential to cause oxidative stress and induce cellular damage to the cancerous cells for the desired effect. Unfortunately, the administration of chemotherapy also damages healthy tissues, inducing a series of unwanted short-term adverse effects such as severe fatigue, nausea, vomiting, diarrhea, headaches, and alopecia, as well as long-term side effects such as organ damage, secondary malignancies (e.g., myelodysplastic syndromes), and neurotoxicity.³⁵

Targeted Therapy and Immunotherapy:

The second arm of intervention in leukemias consists of immunotherapies, which can be systemic and targeted as well. Immunotherapies, often used alongside chemotherapy, are a form of therapeutic cancer intervention in which the body's immune cells and antibodies are used to eliminate pathogenic cells.³⁶ The most common immunotherapies are chimeric antigen receptor (CAR) T-cell therapy, natural killer-mediated immunotherapy with specific targeted mechanisms, and monoclonal antibodies, including immune checkpoint inhibitors, whereas the targeted therapy with tyrosine kinase inhibitors is not considered to be an immunotherapy.³⁶

Chimeric antigen receptor (CAR) T-cell therapy is a targeted immunotherapy that works by attaching a ligand to a receptor for the purposes of disrupting cancer-causing processes, and then it is able to target specific cells with toxic components.²¹ The efficacy of CAR T-cell therapy is notably threatened by a phenomenon known as "low-antigen escape," which means the target is not immunogenic enough to be destroyed by the T-cells delivered.³⁷ It can be affected by the on-target, off-tumor toxicity as well, highlighting major deficiencies in utilizing CAR T-cell therapy in leukemic interventions.

The other forms of immunotherapies are natural killer (NK)-mediated immunotherapies, which are meaningful as they have the ability to detect cells that lack MHC class I molecules. This trait could allow the potential pairing of the usage of natural killer (NK)-mediated immunotherapies and CRISPR-Cas genome editing for targeted killing of cancerous cells that remain after allo-SCT. But their use can be limited due to their potential to induce hyperstimulated activation and thereby weaken the cytotoxic functions of natural killer (NK) cells.^{38,39} This functional deficiency hinders NK cells from performing their normal immunoregulatory functions, thereby predisposing the body to infections. This leads to a potential path to study a combination of therapeutic interventions in blood cancers, in order to make up for the limitations of one therapy with the advantages of another.

Monoclonal antibodies (mAbs) are another form of targeted systemic immunotherapy in leukemias. The mAbs are glycoproteins targeting specific surface antigens and molecules on tumor cells.³⁶ They are divided into four major categories: murine (omab), chimeric (xiomab), humanized (zumab), and fully human (umab) mAbs.³⁶ Nearly half of all antibodies are used in therapeutic cancer interventions.⁴⁰ MABs have a variety of methods by which they function, including blocking receptor-ligand binding and inhibiting cell proliferation. However, the lack of cost-effectiveness makes therapeutic antibodies unconventional, as they limit access to a wider patient base.³⁶

Immune checkpoint inhibitors (ICIs) are a distinct type of monoclonal antibodies (mAbs) that regulate immune cell activation for an anti-tumor response and combat the self-immunity of certain cancer cell types. These cancer cells would otherwise evade destruction by exploiting the body's natural immune checkpoints of preventing autoimmune attack.⁴¹ ICIs provide a more promising and specialized therapeutic intervention, yet their vulnerability to immune escape by leukemia cells and the adverse events from possible autoimmune attack

against the body's own tissues pose an obstacle to their incorporation.⁴¹

Tyrosine kinase inhibitors (TKIs) are small molecules that target and disrupt signaling pathways that allow malignant cell proliferation.⁴² Molecular changes made by TKIs can lead to cell death via various pathways.⁴² They have been used for the last twenty-five years and have been the cornerstone of management of chronic myeloid and chronic lymphocytic leukemias, which are the hematologic malignancies heavily dependent on tyrosine kinases for cellular proliferation and survival. But many of these TKIs are not selective and may inhibit multiple kinases, thereby causing adverse events related to organ damage from "off-target" effects. The limiting factor can also be the adverse events resulting from the "on-target" effects due to excessive inhibition of the tyrosine kinases. These can be in the form of gastrointestinal, cardiac, and skin complications, which can cause short-term or long-term damage.

Radiation Therapy:

Radiation therapy (RT) is another therapeutic intervention specifically utilized for certain types of leukemias like ALL, albeit it is a less frequently indicated treatment for leukemias overall. RT utilizes high-energy beams to damage cancerous cells in leukemia. RT has been used as a bridge therapy to complement alternative cancer interventions such as chimeric antigen receptor (CAR) T cell therapy in certain leukemias, such as ALL, where it sensitizes tumor cells to be attacked by T-cells.⁴³ While it is effective in damaging cancerous cells, it can unfortunately cause an adverse reaction to healthy tissues as well, creating a paradoxical response. For example, in CAR-T cell therapy, if the RT dose is overtly robust, it can induce early activation of T-cells, thereby causing destruction of those very immune cells that were supposed to protect against cancerous cells.⁴³ This has become a limitation in incorporating RT during therapeutic interventions.

Stem Cell Transplantation: Advanced Therapeutic Potential in Leukemias:

Table 2: Efficacy is measured by the 5-year OS of various therapies for ALL. The table below, created in BioRender, reviews the leading therapies for leukemias, including chemotherapy, immunotherapy, radiation therapy, stem cell transplantation, and targeted therapy. While ALL is most common in pediatric populations, data for immunotherapy usage in ALL were limited to adult populations due to the unavailability of pediatric populations. Overall survival (OS). Leukemia-free survival (LFS). Data were acquired from a variety of sources.⁴⁷⁻⁵⁰

Current Therapies to Treat Acute Lymphoblastic Leukemia (ALL)

Type of Therapy	Mechanism of Action	Advantages	Disadvantages	Data collection population	Efficacy (5-year OS)	References
Chemotherapy	DNA damage induced by cytotoxic drugs	Rapid drug onset	Healthy cell damage, drug resistance, dosage selection difficulty	Pediatric	85-90%	[47 - 50]
Immunotherapy	Manipulation of immune system to facilitate cancer cell destruction	Sustained anti-tumor effects	Immune related adverse events (irAEs), hyper-progression, reactivation of disease	Adults	65-70% (using 67.8% 5-year LFS)	[47 - 50]
Radiation Therapy	DNA damage caused by X-rays	Localized control of cancer	Tissue damage, low blood counts	Pediatric	70-80%	[47 - 50]
Stem Cell Transplantation (SCT)	Replacement of dysfunctional bone marrow with healthy HSCs	Tissue regeneration, avoidance of invasive surgery	Tumorigenesis, graft versus host disease (GVHD), drug toxicity	Pediatric	70-90%	[47 - 50]
Targeted Therapy (TKIs, venetoclax, etc.)	Targeting of specific molecules to slow cell growth	High specificity, reduced toxicity, oral administration	Treatment resistance, dermatologic adverse events (dAEs)	Pediatric	>80%	[47 - 50]

Given the limitations of the aforementioned therapies for leukemias, it is key to explore the treatment modality of stem cell transplantation (SCT) as one of the leading alternatives, especially in refractory and relapsed cases, as well as for those cases with an adverse cytogenetic profile.^{44,45} Stem cell transplantation offers the potential for long-lasting remission as well as cure in leukemias, making it the second-line treatment for certain refractory and relapsed cases of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).⁴⁶ “The use of bone marrow transplantation (a type of SCT) from compatible donors is the most effective treatment for CML” because alternative therapies might not work effectively and cause severe side effects to patients.⁴⁶

Autologous and Allogeneic SCT as Therapeutic Interventions in Leukemias, Hemoglobinopathies, and Inherited Red Blood Cell Disorders:

There are two distinct types of stem cell treatment based on their source: autologous and allogeneic. Autologous stem cell transplantation (auto-SCT) consists of transplanting the individual's own stem cells, in contrast to allogeneic stem cell transplantation (allo-SCT), where the stem cells from a matched donor are obtained from either a family member or an individual unrelated to the patient. Their indications of use in leukemia are based on the patient profile, type of leukemia, and the response to available therapies.⁴⁶

In autologous stem cell transplant therapy, the patient's intrinsic stem cells are collected and treated *ex vivo* before re-infusion into the patient's own body to treat a condition. This can only be done if their bone marrow is healthy enough to obtain stem cells. Auto-SCT is more accessible, timely, and has the advantage of better acceptance of transplanted tissue without the need for any HLA matching. But auto-SCT does pose a risk of relapsed leukemia due to possible residual disease, thereby limiting the progression-free survival of affected patients.²² Hence, for its effective use therapeutically to treat the disease condition, editing with genetic engineering technologies like the CRISPR-Cas9 endonuclease system can facilitate “silencing” or “reactivating” a gene to make it functional. Thereafter, these edited intrinsic stem cells can be reinfused in the patient to treat their leukemia, which is considered an autologous transplant.³³ While this process of “cleaning out the residual disease from harvested stem cells using gene edits” is still undergoing clinical trials for auto-SCT in leukemias, it has been successfully carried out for patients with inherited red blood cell disorders—hemoglobinopathies like sickle cell disease. An example is Casgevy, which is the first FDA-approved CRISPR-based treatment for severe sickle cell disease and beta-thalassemia; this treatment has been successful in clinical use as of 2023. The disadvantage of incorporating autologous stem cell transplantation in the therapeutic protocol for leukemia is that the intrinsic stem cells could be cancerous in the affected individuals with leukemia, thereby potentially causing relapse of the disease, while not helping replenish hematopoietic (blood-producing) cells easily.⁵¹ The other major limitation is the cost related to this modality of treatment, thereby creating inequitable access.

An allo-SCT is the transplantation of stem cells from another individual—related or unrelated—being transfused into the patient's body to treat the blood disorder. Such transplantation needs to be matched for HLA, the immune-recognition marker, and offers the advantage of being more efficacious in inducing remission due to the graft-versus-leukemia (GVL) effect. This makes it a more sought-after treatment of certain leukemias in comparison to autologous.^{24,46} Allo-SCTs may facilitate longer remission and prevent progression of the disease, depending on the specific leukemia and other individual risk factors.^{22,23} However, there are limiting factors for allogeneic stem cell transplantation since close compatibility of the donor's HLA with the patient's is necessary for a successful transplant outcome. It is also imperative to align HLA closely to help prevent rejection of the transplanted tissue for ameliorating the affected patient's condition. This would also help minimize the risk of an adverse immune response mounted by the transplanted donor tissue against the patient's normal tissues, graft-versus-host disease (GVHD).^{24,51} But as evidence shows, the process of securing a fully matched stem cell donor can be labor-intensive and time-consuming, which can delay the chances of a patient's medical condition improving. In the meantime, affected patients can continue to worsen and possibly suffer complications from their disease. It takes an average of 52 days to get to the matched donor information session for beginning the process of allo-SCT, which is longer than the average duration of an auto-SCT.⁵²

This paper investigates the progress made in the precision delivery of effective allogeneic stem cells using the CRISPR-Cas system genome editing to modify HLA genes and to facilitate successful acceptance by the recipient.⁵³ It is worthwhile to consider the potential success of genetic engineering techniques like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas genome editing in precision medicine for abrogating HLA genes. These HLA-deficient or HLA-negative donor stem cells or lymphocytes become hypoinmunogenic, thereby evading the host immune system's detection.^{26,54} The resulting successful allogeneic stem cell transplants potentially induce a durable remission of the leukemia and lead to event-free survival in the affected patients.

Gene Editing Biotechnologies: TALENs, ZFNs, and CRISPR-Cas:

First, we delve into the uses and limitations of ZFNs and TALENs in this realm. Then, we will discuss the advantages and detailed processes of utilizing the CRISPR-Cas genome editing tool to help in rendering “universal” donor stem cells. ZFNs and TALENs are programmable nucleases that consist of a nonspecific DNA cleavage portion linked to sequence-specific DNA-binding modules.²⁹ They induce these edits in the genes by making double-stranded breaks in the DNA, which thereafter stimulate DNA repair mechanisms in the form of homology-directed repair (HDR) at specific genomic locations or non-homologous end-joining (NHEJ); these mechanisms are also called “gene-knockins” and “gene knock-outs”, respectively.⁵⁴ Although the flexibility of this targeted

approach has brought genome engineering to the forefront of therapeutics in leukemias, they are more time-consuming, costly to manufacture, and lack site-specificity at times. This is where more efficient delivery systems like the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats - Cas endonuclease) gene editing system have been a better alternative comparatively for targeted genetic modifications.²⁹

Introduction of the CRISPR-Cas Gene Editing Tool:

CRISPR-Cas9 is an innovative gene editing tool functioning as a pair of molecular scissors that utilizes a single-guide RNA (sgRNA) and Cas9 protein duplex to edit DNA.³⁰ This is an important part of the adaptive immune system in prokaryotes to direct the precise removal or insertion of DNA fragments with a bacterial enzyme. The bacterial CRISPR locus was first described by Francisco Mojica, and it is made of viral or plasmid DNA that previously infected the microbes, found between the array of short palindromic sequences, called “spacers.”⁵⁵ It was patented just over a decade ago by E. Charpentier and J.A. Doudna, who repurposed the type II CRISPR/Cas9 system for use in therapeutics. This gene editing tool has its roots in an adaptive bacterial immune system—bacteria loading a portion of the attacking viral DNA into their own genome as “memory” for the future and for prevention of re-infection with the virus. Target recognition by the Cas9 protein, an endonuclease (“the molecular scissors”), requires an identifying sequence known as protospacer adjacent motif (PAM), “the postal address,” which is upstream of the crRNA-binding region. This allows the CRISPR-Cas complex to be adaptive as per the necessities of gene editing by redesigning the crRNA to cleave any DNA sequence.⁵⁶ Compared to ZFNs and TALENs, it is more effective and simpler to produce with a short turnaround time, thereby reducing the economic burdens involved.^{24,29} The different delivery methods for CRISPR-Cas9 include viral gene delivery (e.g., adenovirus, lentivirus, retrovirus) and non-viral gene delivery systems such as DNA electroporation, transposons, nanocarriers, and cell-squeeze techniques involving various bioactive materials.²⁹ The details of these delivery systems, including uses and limitations, are beyond the scope of this paper.

CRISPR-Cas in Stem Cell Transplantation: Advantages and Limitations:

CRISPR has evolved to help delete genes of HLA Class I and II antigens, which make the donor stem cells “escape” the patient’s immune system, becoming more acceptable to the patient’s body; Figure 3 depicts this HLA cleavage and its intended effect.⁵⁷ As the rejection of foreign stem cells can be prevented, it precludes the need for closely matching the HLA as well.^{30,32,54} This facilitates such stem cells from the donor to become more “universal” in nature and ready “off-the-shelf” for the purpose of effectively treating rare and refractory chemo-radiation-resistant leukemias while minimizing the wait time to find HLA-matched donors. The additional uses of CRISPR in the arena of stem cell transplantation are the prevention of automatic destruction of such HLA-deficient stem cells by the natural killer (NK)-cell response, which does this

by overexpressing NK-inhibitory ligands.^{38,39} The other method where CRISPR is useful in allogeneic stem cell transplants is the T-cell receptor “gene-deletion” in donor T-lymphocytes, which can prevent them from launching an attack on the recipient’s foreign cells, thereby preventing graft-versus-host disease and related adverse events.

HLA Class I B2M gene cleavage via CRISPR-Cas9

Targeting B2M gene to switch off HLA expression in HLA Class I

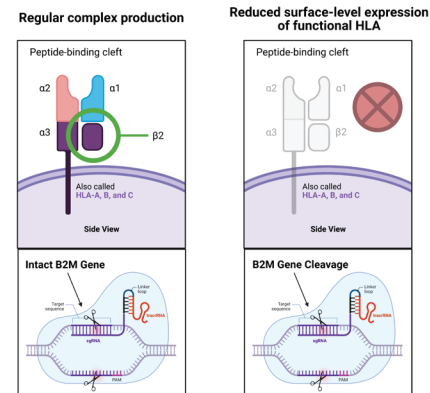


Figure 3: A visual depiction of the potential to reduce surface-level expression of functional Class I HLA by targeting the beta-2-microglobulin gene via CRISPR-Cas9. This figure was made in BioRender and adapted from a figure made in collaboration between BioRender and the Doudna lab.⁵⁷

The CRISPR-Cas system has provided a quicker and simpler solution to manipulate the genome for therapeutic benefits, but it still has several limitations that need to be evaluated further and alleviated.³⁰ Just like we need to discuss the indications and advantages of the CRISPR-Cas system, we need to note the limitations of this gene-editing tool as well. The limitations can be the possibility of tumorigenesis through triggering of a p53-dependent DNA damage response that induces cell death and apoptosis of such affected cells with functional p53, whereas the ones with p53 mutations get naturally filtered out for survival and uncontrolled multiplication.²⁹ This can result in the suppression of the gene-editing tool itself due to the arrest of the cell cycle and division, which prevents CRISPR-related repair mechanisms from being successful.²⁹ There are also chances of genome editing by CRISPR being rendered ineffective due to an adaptive immune reaction against it by the host’s immune system. In addition, there is a higher risk of off-target mutations due to genomic instability and unintentional disruption of other genes, which may need a “switch-off” mechanism. There is promise in single-base editing or substitution to minimize the off-target effects, as well as disruption of genes, causing inadvertent mutations during repair.⁵⁸

Conclusion

Hematopoietic stem cell transplantation (HSCT) has largely been successful in treating certain leukemias, the ones that are refractory or resistant to conventional therapeutics, and in cases where those have caused severe adverse effects for the patients. Data shows although the disease burden of leukemias has increased recently, the good news is that overall survival (OS) and event-free survival (EFS) across all leukemias and

patient populations, especially in the pediatric population, have also increased.² We can certainly credit these to novel and safer targeted therapies, immunotherapeutics, and stem cell transplantation in combination with precision medicine and gene-editing technologies. Of the current genetic engineering tools, ZFNs and TALENs have some limitations due to their off-target effects in their practical applications, which makes the CRISPR-Cas system more viable as a therapeutic tool for effective and more specifically targeted delivery in leukemias. Stem cell transplantation, especially allogeneic, has more potential for enduring remission in patients with refractory, relapsed leukemias as compared to autologous, which has limited indications in certain acute myeloid leukemias. The need for close HLA compatibility in allogeneic stem cell transplants for safe and successful transplant outcomes makes it extremely unlikely for many patients to access the limited stem cell donor pool over their lifetime. Their quality of life and survival chances are adversely affected. Hence, the use of gene-editing techniques, especially CRISPR-Cas duplexes to deliver hypo-immunogenic and “universal” allogeneic stem cells in a quicker turnaround time to patients with leukemias, seems extremely promising.

The goal of therapeutic gene editing is to make potentially curative stem cells safer by reducing the off-target effects and associated risks to patients.⁵⁶ These processes are still evolving, with better design of the CRISPR-Cas guide RNA, using a variant of Cas9 nickase (Cas9n) for inducing single-stranded breaks, as well as with single-base and prime editing; this should help better accuracy, obviating the off-target effects. Additionally, the newer technologies of direct *in vivo* gene-editing tools explored in other genetic musculoskeletal and solid tissue tumors can be extrapolated to therapeutic applications in blood cancers and blood cell disorders. However there are some limitations of genetic engineering technologies, like the economic burdens on the healthcare system, differential access to resources in varied settings, and the need for solutions in order to reduce such inequities created from the very high costs of CRISPR-edited stem cells. Moreover, it is necessary to adhere to the ethical regulatory frameworks of the government and entities about genetic modification that may inadvertently cause long-lasting complications in recipients, including tumorigenesis.⁵⁹

In summary, gene-editing precision tools like CRISPR-Cas have kick-started a revolutionary transformation in the treatment options for leukemias and, in the future, have the potential to lead to improved outcomes of better quality of life for such patients in a more equitable manner.

■ AI Acknowledgments

During the preparation of this work, the author used Google AI Overview and the standard version of Grammarly to learn definitions and concepts and improve language readability, respectively. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

■ Acknowledgments

I would like to extend my gratitude to Dr. Hamidreza Shaye and Dr. Lauren Tetz for providing me with support and guidance in writing this review. I would like to thank my mom, Dr. Arathi Shah, for inspiring me to research the potential of using CRISPR-Cas in SCT and aiding me in the creative process.

■ References

1. Daltveit, D. S.; Morgan, E.; Colombet, M.; Steliarova-Foucher, E.; Bendahhou, K.; Marcos-Gragera, R.; Rongshou, Z.; Smith, A.; Wei, H.; Soerjomataram, I. Global Patterns of Leukemia by Subtype, Age, and Sex in 185 Countries in 2022. *Leukemia* **2025**, *39* (2), 412–419. <https://doi.org/10.1038/s41375-024-02452-y>.
2. Du, M.; Chen, W.; Liu, K.; Wang, L.; Hu, Y.; Mao, Y.; Sun, X.; Luo, Y.; Shi, J.; Shao, K.; Huang, H.; Ye, D. The Global Burden of Leukemia and Its Attributable Factors in 204 Countries and Territories: Findings from the Global Burden of Disease 2019 Study and Projections to 2030. *J Oncol* **2022**, *2022*, 1612702. <https://doi.org/10.1155/2022/1612702>.
3. Chennamadhavuni, A.; Iyengar, V.; Mukkamalla, S. K. R.; Shimanovsky, A. Leukemia. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2026.
4. Bispo, J. A. B.; Pinheiro, P. S.; Kobetz, E. K. Epidemiology and Etiology of Leukemia and Lymphoma. *Cold Spring Harb Perspect Med* **2020**, *10* (6), a034819. <https://doi.org/10.1101/cshperspect.a034819>.
5. National Cancer Institute. *Acute Myeloid Leukemia - Cancer Stat Facts*. SEER. <https://seer.cancer.gov/statfacts/html/amyl.html>.
6. Azad, N. A.; Shah, Z. A.; Pandith, A. A.; Rasool, R.; Jeelani, S. Real-Time Quantitative PCR: A Reliable Molecular Diagnostic and Follow-up Tool for “minimal Residual Disease” Assessment in Chronic Myeloid Leukemia. *Biosci Rep* **2018**, *38* (5), BSR20180974. <https://doi.org/10.1042/BSR20180974>.
7. Bains, A.; Luthra, R.; Medeiros, L. J.; Zuo, Z. FLT3 and NPM1 Mutations in Myelodysplastic Syndromes: Frequency and Potential Value for Predicting Progression to Acute Myeloid Leukemia. *Am J Clin Pathol* **2011**, *135* (1), 62–69. <https://doi.org/10.1309/AJC-PEI9XU8PYBCIO>.
8. Blanco, G.; Puiggros, A.; Baliakas, P.; Athanasiadou, A.; García-Malo, M. D.; Collado, R.; Xochelli, A.; Rodríguez-Rivera, M.; Ortega, M.; Calasanz, M. J.; Luño, E.; Vargas, M. T.; Grau, J.; Martínez-Laperche, C.; Valiente, A.; Cervera, J.; Anagnostopoulos, A.; Gimeno, E.; Abella, E.; Stalika, E.; Hernández-Rivas, J. M.; Ortuño, F. J.; Robles, D.; Ferrer, A.; Ivars, D.; González, M.; Bosch, F.; Abrisqueta, P.; Stamatopoulos, K.; Espinet, B.; Blanco, G.; Puiggros, A.; Baliakas, P.; Athanasiadou, A.; García-Malo, M. D.; Collado, R.; Xochelli, A.; Rodríguez-Rivera, M.; Ortega, M.; Calasanz, M. J.; Luño, E.; Vargas, M. T.; Grau, J.; Martínez-Laperche, C.; Valiente, A.; Cervera, J.; Anagnostopoulos, A.; Gimeno, E.; Abella, E.; Stalika, E.; Hernández-Rivas, J. M.; Ortuño, F. J.; Robles, D.; Ferrer, A.; Ivars, D.; González, M.; Bosch, F.; Abrisqueta, P.; Stamatopoulos, K.; Espinet, B. Karyotypic Complexity Rather than Chromosome 8 Abnormalities Aggravates the Outcome of Chronic Lymphocytic Leukemia Patients with TP53 Aberrations. *Oncotarget* **2016**, *7* (49), 80916–80924. <https://doi.org/10.18632/oncotarget.13106>.
9. Boer, J. M.; Steeghs, E. M. P.; Marchante, J. R. M.; Boeree, A.; Beaudoin, J. J.; Beverloo, H. B.; Kuiper, R. P.; Escherich, G.; Van Der Velden, V. H. J.; Van Der Schoot, C. E.; De Groot-Kruseman, H. A.; Pieters, R.; Den Boer, M. L. Tyrosine Kinase Fusion Genes in Pediatric *BCR-ABL1* -like Acute Lymphoblastic Leuke-

- mia. *Oncotarget* **2017**, *8* (3), 4618–4628. <https://doi.org/10.18632/oncotarget.13492>.
10. Bucchieri, V.; Barreto, W. G.; Fogliatto, L. M.; Capra, M.; Marchiani, M.; Rocha, V. Prognostic and Therapeutic Stratification in CLL: Focus on 17p Deletion and P53 Mutation. *Ann Hematol* **2018**, *97* (12), 2269–2278. <https://doi.org/10.1007/s00277-018-3503-6>.
 11. Langabeer, S. E.; McCarron, S. L.; Kelly, J.; Krawczyk, J.; McPherson, S.; Perera, K.; Murphy, P. T. Chronic Myeloid Leukemia with E19a2 BCR-ABL1 Transcripts and Marked Thrombocytosis: The Role of Molecular Monitoring. *Case Reports in Hematology* **2012**, *2012*, 1–3. <https://doi.org/10.1155/2012/458716>.
 12. Morris, C. M. Chronic Myeloid Leukemia: Cytogenetic Methods and Applications for Diagnosis and Treatment. In *Cancer Cytogenetics*; Campbell, L. J., Ed.; Humana Press: Totowa, NJ, **2011**; Vol. 730, pp 33–61. https://doi.org/10.1007/978-1-61779-074-4_4.
 13. Schneider, F.; Hoster, E.; Schneider, S.; Dufour, A.; Benthaus, T.; Kakadia, P. M.; Bohlander, S. K.; Braess, J.; Heinecke, A.; Sauerland, M. C.; Berdel, W. E.; Buechner, T.; Woermann, B. J.; Feuring-Buske, M.; Buske, C.; Creutzig, U.; Thiede, C.; Zwaan, M. C.; Van Den Heuvel-Eibrink, M. M.; Reinhardt, D.; Hiddemann, W.; Spiekermann, K. Age-Dependent Frequencies of NPM1 Mutations and FLT3-ITD in Patients with Normal Karyotype AML (NK-AML). *Ann Hematol* **2012**, *91* (1), 9–18. <https://doi.org/10.1007/s00277-011-1280-6>.
 14. Zainuddin, N.; Murray, F.; Kanduri, M.; Gunnarsson, R.; Smedby, K. E.; Enblad, G.; Jurlander, J.; Juliusson, G.; Rosenquist, R. TP53 Mutations Are Infrequent in Newly Diagnosed Chronic Lymphocytic Leukemia. *Leukemia Research* **2011**, *35* (2), 272–274. <https://doi.org/10.1016/j.leukres.2010.08.023>.
 15. Zhang, L.; Habeebu, S. S. M.; Li, W. Prognostic and Predictive Biomarkers in Precursor B-Cell Acute Lymphoblastic Leukemia. In *Leukemia*; Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, USA, Weijie, L., Eds.; Exon Publications, **2022**; pp 165–193. <https://doi.org/10.36255/exon-publications-leukemia-biomarkers-lymphoblastic-leukemia>.
 16. Tripathi, A. K.; Chuda, R. Laboratory Evaluation of Acute Leukemia. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), **2026**.
 17. Choi, H.-S.; Kim, B. S.; Yoon, S.; Oh, S.-O.; Lee, D. Leukemic Stem Cells and Hematological Malignancies. *Int J Mol Sci* **2024**, *25* (12), 6639. <https://doi.org/10.3390/ijms25126639>.
 18. De Boer, B.; Sheveleva, S.; Apelt, K.; Vellenga, E.; Mulder, A. B.; Huls, G.; Jacob Schuringa, J. The IL1-IL1RAP Axis Plays an Important Role in the Inflammatory Leukemic Niche That Favors Acute Myeloid Leukemia Proliferation over Normal Hematopoiesis. *Haematologica* **2021**, *106* (12), 3067–3078. <https://doi.org/10.3324/haematol.2020.254987>.
 19. Hamid, G. A. Acute Leukemia Clinical Presentation. In *Leukemia*; IntechOpen, **2013**. <https://doi.org/10.5772/53531>.
 20. Khwaja, A.; Bjorkholm, M.; Gale, R. E.; Levine, R. L.; Jordan, C. T.; Ehninger, G.; Bloomfield, C. D.; Estey, E.; Burnett, A.; Cornelissen, J. J.; Scheinberg, D. A.; Bouscary, D.; Linch, D. C. Acute Myeloid Leukaemia. *Nat Rev Dis Primers* **2016**, *2*, 16010. <https://doi.org/10.1038/nrdp.2016.10>.
 21. Debela, D. T.; Muzazu, S. G.; Heraro, K. D.; Ndalama, M. T.; Mesele, B. W.; Haile, D. C.; Kitui, S. K.; Manyazewal, T. New Approaches and Procedures for Cancer Treatment: Current Perspectives. *SAGE Open Med* **2021**, *9*, 20503121211034366. <https://doi.org/10.1177/20503121211034366>.
 22. Ye, W.; Wu, X.; Zhao, R.; Jin, X.; Li, H.; Qu, Y.; Ji, J.; Liu, Z. Comparison of Allo-SCT, Auto-SCT and Chemotherapy for the Treatment of Patients with Low- or Intermediate-Risk Acute Myeloid Leukemia: A Network Meta-Analysis. *Stem Cell Res Ther* **2024**, *15*, 153. <https://doi.org/10.1186/s13287-024-03766-5>.
 23. Chevillon, F.; Dhédin, N.; Boissel, N. Allogeneic Hematopoietic Stem Cell Transplantation in Adults with Acute Lymphoblastic Leukemia: Evolving Indications and Modalities in Shifting Landscape. *Leuk Lymphoma* **2026**, *67* (2), 282–294. <https://doi.org/10.1080/10428194.2025.2584685>.
 24. Mangum, D. S.; Caywood, E. A Clinician's Guide to HLA Matching in Allogeneic Hematopoietic Stem Cell Transplant. *Hum Immunol* **2022**, *83* (10), 687–694. <https://doi.org/10.1016/j.humimm.2022.03.002>.
 25. Medhasi, S.; Chantratita, N. Human Leukocyte Antigen (HLA) System: Genetics and Association with Bacterial and Viral Infections. *J Immunol Res* **2022**, *2022*, 9710376. <https://doi.org/10.1155/2022/9710376>.
 26. Montgomery, R. A.; Tatapudi, V. S.; Leffell, M. S.; Zachary, A. A. HLA in Transplantation. *Nat Rev Nephrol* **2018**, *14* (9), 558–570. <https://doi.org/10.1038/s41581-018-0039-x>.
 27. Budeus, B.; Álvaro-Benito, M.; Crivello, P. HLA-DM and HLA-DO Interplay for the Peptide Editing of HLA Class II in Healthy Tissues and Leukemia. *Best Pract Res Clin Haematol* **2024**, *37* (2), 101561. <https://doi.org/10.1016/j.beha.2024.101561>.
 28. Bayarsaikhan, D.; Bayarsaikhan, G.; Lee, B. Recent Advances in Stem Cells and Gene Editing: Drug Discovery and Therapeutics. *Prog Mol Biol Transl Sci* **2021**, *181*, 231–269. <https://doi.org/10.1016/bs.pmbts.2021.01.019>.
 29. Gaj, T.; Gersbach, C. A.; Barbas, C. F. ZFN, TALEN, and CRISPR/Cas-Based Methods for Genome Engineering. *Trends Biotechnol* **2013**, *31* (7), 397–405. <https://doi.org/10.1016/j.tibtech.2013.04.004>.
 30. Lee, J.; Sheen, J. H.; Lim, O.; Lee, Y.; Ryu, J.; Shin, D.; Kim, Y. Y.; Kim, M. Abrogation of HLA Surface Expression Using CRISPR/Cas9 Genome Editing: A Step toward Universal T Cell Therapy. *Sci Rep* **2020**, *10* (1), 17753. <https://doi.org/10.1038/s41598-020-74772-9>.
 31. Lyu, Z.; Niu, S.; Fang, Y.; Chen, Y.; Li, Y.-R.; Yang, L. Addressing Graft-versus-Host Disease in Allogeneic Cell-Based Immunotherapy for Cancer. *Exp Hematol Oncol* **2025**, *14* (1), 66. <https://doi.org/10.1186/s40164-025-00654-3>.
 32. Lanza, R.; Russell, D. W.; Nagy, A. Engineering Universal Cells That Evade Immune Detection. *Nat Rev Immunol* **2019**, *19* (12), 723–733. <https://doi.org/10.1038/s41577-019-0200-1>.
 33. Dever, D. P.; Bak, R. O.; Reinisch, A.; Camarena, J.; Washington, G.; Nicolas, C. E.; Pavel-Dinu, M.; Saxena, N.; Wilkens, A. B.; Mantri, S.; Uchida, N.; Hendel, A.; Narla, A.; Majeti, R.; Weinberg, K. I.; Porteus, M. H. CRISPR/Cas9 β -Globin Gene Targeting in Human Hematopoietic Stem Cells. *Nature* **2016**, *539* (7629), 384–389. <https://doi.org/10.1038/nature20134>.
 34. Wendt, A. S.; Brintrup, J.; Waid, J. L.; Kader, A.; Lambrecht, N. J.; Gabrysch, S. Thalassemia and Hemoglobinopathy Prevalence in a Community-Based Sample in Sylhet, Bangladesh. *Orphanet J Rare Dis* **2023**, *18* (1), 192. <https://doi.org/10.1186/s13023-023-02821-3>.
 35. Śliwa-Tytko, P.; Kaczmarek, A.; Lejman, M.; Zawitkowska, J. Neurotoxicity Associated with Treatment of Acute Lymphoblastic Leukemia Chemotherapy and Immunotherapy. *Int J Mol Sci* **2022**, *23* (10), 5515. <https://doi.org/10.3390/ijms23105515>.
 36. Damián-Blanco, P.; Ahuexoteco-Sánchez, S.; Carbajal-Gallardo, A. A.; Coctecon-Chávelas, F. C.; Rodríguez-Nava, C.; Vences-Velázquez, A.; Medina-Flores, Y.; Mata-Ruiz, O.; Lloret-Sánchez, L.; Cortés-Sarabia, K. Use of Monoclonal Antibodies in Cancer Immunotherapy: Types and Mechanisms of

- Action. *Bol Med Hosp Infant Mex* **2023**, *80* (3), 153–164. <https://doi.org/10.24875/BMHIM.23000123>.
37. Bartoszewski, E.; Tota, M.; Kislewska, M.; Skowron, I.; Sebastianka, K.; Stefaniak, O.; Molik, K.; Rubin, J.; Kraska, K.; Choromańska, A. Overcoming Antigen Escape and T-Cell Exhaustion in CAR-T Therapy for Leukemia. *Cells* **2024**, *13* (18), 1596. <https://doi.org/10.3390/cells13181596>.
 38. Iyoda, T.; Yamasaki, S.; Ueda, S.; Shimizu, K.; Fujii, S.-I. Natural Killer T and Natural Killer Cell-Based Immunotherapy Strategies Targeting Cancer. *Biomolecules* **2023**, *13* (2), 348. <https://doi.org/10.3390/biom13020348>.
 39. Lian, G.; Mak, T. S.-K.; Yu, X.; Lan, H.-Y. Challenges and Recent Advances in NK Cell-Targeted Immunotherapies in Solid Tumors. *Int J Mol Sci* **2021**, *23* (1), 164. <https://doi.org/10.3390/ijms23010164>.
 40. Delgado, M.; Garcia-Sanz, J. A. Therapeutic Monoclonal Antibodies against Cancer: Present and Future. *Cells* **2023**, *12* (24), 2837. <https://doi.org/10.3390/cells12242837>.
 41. Jo, W.; Won, T.; Daoud, A.; Čiháková, D. Immune Checkpoint Inhibitors Associated Cardiovascular Immune-Related Adverse Events. *Front Immunol* **2024**, *15*, 1340373. <https://doi.org/10.3389/fimmu.2024.1340373>.
 42. Shyam Sunder, S.; Sharma, U. C.; Pokharel, S. Adverse Effects of Tyrosine Kinase Inhibitors in Cancer Therapy: Pathophysiology, Mechanisms and Clinical Management. *Signal Transduct Target Ther* **2023**, *8* (1), 262. <https://doi.org/10.1038/s41392-023-01469-6>.
 43. Sugita, M.; Yamazaki, T.; Alhomoud, M.; Martinet, J.; Latouche, J.-B.; Golden, E.; Boyer, O.; Van Besien, K.; Formenti, S. C.; Galluzzi, L.; Guzman, M. L. Radiation Therapy Improves CAR T Cell Activity in Acute Lymphoblastic Leukemia. *Cell Death Dis* **2023**, *14* (5), 305. <https://doi.org/10.1038/s41419-023-05829-6>.
 44. Halaburda, K.; Labopin, M.; Mailhol, A.; Socié, G.; Craddock, C.; Aljurf, M.; Beelen, D.; Cornelissen, J. J.; Bourhis, J.-H.; Labussière-Wallet, H.; Blaise, D.; Gedde-Dahl, T.; Gillece, M.; Yakoub-Agha, I.; Mufti, G.; Esteve, J.; Mohty, M.; Nagler, A. Allogeneic Stem Cell Transplantation in Second Complete Remission for Core Binding Factor Acute Myeloid Leukemia: A Study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Haematologica* **2020**, *105* (6), 1723–1730. <https://doi.org/10.3324/haematol.2019.222810>.
 45. Arias-Espinosa, L.; Acosta-Medina, A. A.; Vargas-España, A.; Fuentes-Martin, V.; Colunga-Pedraza, P. R.; Hawing-Zarate, J. A.; Leon, A. G.-D.; Soto-Mota, A.; Pacheco-Gutierrez, G.; Vargas-Serafin, C.; Barrera-Lumbreras, G.; Bournon, C. Acute Leukemia Relapse after Hematopoietic Stem Cell Transplantation: The Good, the Bad, and the Ugly of Isolated Extramedullary Relapse in a Latin American Population. *Transplant Cell Ther* **2023**, *29* (8), 510.e1–510.e9. <https://doi.org/10.1016/j.jctc.2023.05.006>.
 46. Dessie, G.; Derbew Molla, M.; Shibabaw, T.; Ayelign, B. Role of Stem-Cell Transplantation in Leukemia Treatment. *Stem Cells Cloning* **2020**, *13*, 67–77. <https://doi.org/10.2147/SCCAA.S262880>.
 47. Boissel, N. Where Do Immunotherapies Stand in the Management of Acute Leukemia in Adults? *Hematology* **2025**, *2025* (1), 342–352. <https://doi.org/10.1182/hematology.2025000723>.
 48. Kantarjian, H.; Jabbour, E. Adult Acute Lymphoblastic Leukemia: 2025 Update on Diagnosis, Therapy, and Monitoring. *Am J Hematol* **2025**, *100* (7), 1205–1231. <https://doi.org/10.1002/ajh.27708>.
 49. Miller, K. D.; Nogueira, L.; Devasia, T.; Mariotto, A. B.; Yabroff, K. R.; Jemal, A.; Kramer, J.; Siegel, R. L. Cancer Treatment and Survivorship Statistics, 2022. *CA Cancer J Clinicians* **2022**, *72* (5), 409–436. <https://doi.org/10.3322/caac.21731>.
 50. Wagle, N. S.; Nogueira, L.; Devasia, T. P.; Mariotto, A. B.; Yabroff, K. R.; Islami, F.; Jemal, A.; Alteri, R.; Ganz, P. A.; Siegel, R. L. Cancer Treatment and Survivorship Statistics, 2025. *CA Cancer J Clin* **2025**, *75* (4), 308–340. <https://doi.org/10.3322/caac.70011>.
 51. Champlin, R. Selection of Autologous or Allogeneic Transplantation. In *Holland-Frei Cancer Medicine. 6th edition*; BC Decker, 2003.
 52. Worel, N.; Mahmoud Aljurf; Anthias, C.; Buser, A.; Cody, M.; Fechter, M.; Galeano, S.; Greinix, H.; Kisch, A. M.; Koh, M.; Thilo Mengling; Grazia Nicoloso; Dietger Niederwieser; Pulsipher, M. A.; Seber, A.; Shaw, B. E.; Stefanski, H. E.; Switzer, G. E.; Szer, J.; Walraven, van. Suitability of Hematopoietic Cell Donors: Updated Consensus Recommendations from the WBMT Standing Committee on Donor Issues. **2022**, *9* (8), e605–e614. [https://doi.org/10.1016/s2352-3026\(22\)00184-3](https://doi.org/10.1016/s2352-3026(22)00184-3).
 53. Deuse, T.; Hu, X.; Gravina, A.; Wang, D.; Tediashvili, G.; De, C.; Thayer, W. O.; Wahl, A.; Garcia, J. V.; Reichenspurner, H.; Davis, M. M.; Lanier, L. L.; Schrepfer, S. Hypoimmunogenic Derivatives of Induced Pluripotent Stem Cells Evade Immune Rejection in Fully Immunocompetent Allogeneic Recipients. *Nat Biotechnol* **2019**, *37* (3), 252–258. <https://doi.org/10.1038/s41587-019-0016-3>.
 54. Kim, J.; Nam, Y.; Jeon, D.; Choi, Y.; Choi, S.; Hong, C. P.; Kim, S.; Jung, H.; Park, N.; Sohn, Y.; Rim, Y. A.; Ju, J. H. Generation of Hypoimmunogenic Universal iPS Cells through HLA-Type Gene Knockout. *Exp Mol Med* **2025**, *57* (3), 686–699. <https://doi.org/10.1038/s12276-025-01422-3>.
 55. Uddin, F.; Rudin, C. M.; Sen, T. CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. *Front Oncol* **2020**, *10*, 1387. <https://doi.org/10.3389/fonc.2020.01387>.
 56. Daniel-Moreno, A.; Lamsfus-Calle, A.; Raju, J.; Antony, J. S.; Handgretinger, R.; Mezger, M. CRISPR/Cas9-Modified Hematopoietic Stem Cells—Present and Future Perspectives for Stem Cell Transplantation. *Bone Marrow Transplant* **2019**, *54* (12), 1940–1950. <https://doi.org/10.1038/s41409-019-0510-8>.
 57. *A tool for genome editing: CRISPR-Cas9*. <https://www.biorender.com/blog/nobel-prize-chemistry-2020> (accessed 2026-06-11).
 58. Ma, X.; Chen, X.; Jin, Y.; Ge, W.; Wang, W.; Kong, L.; Ji, J.; Guo, X.; Huang, J.; Feng, X.-H.; Fu, J.; Zhu, S. Small Molecules Promote CRISPR-Cpf1-Mediated Genome Editing in Human Pluripotent Stem Cells. *Nat Commun* **2018**, *9* (1), 1303. <https://doi.org/10.1038/s41467-018-03760-5>.
 59. De Novellis, D.; Cacace, F.; Caprioli, V.; Wierda, W. G.; Mahadeo, K. M.; Tambaro, F. P. The TKI Era in Chronic Leukemias. *Pharmaceutics* **2021**, *13* (12), 2201. <https://doi.org/10.3390/pharmaceutics13122201>.

■ Authors

Rishabh Shah is a senior at Westlake High School. He is passionate about oncogenetics and leads local stem cell drives with DKMS and NMDP to diversify the global donor registry. He hopes to major in biochemistry and the classics in the future. This review explores his interest in using gene editing tools to make stem cell transplantation safer and more accessible.