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Genetic breakthrough in medicine: an analysis of Exa-cel, the first FDA-approved CRISPR treatment for sickle cell disease

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ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**GENETIC BREAKTHROUGH IN MEDICINE:
AN ANALYSIS OF EXA-CEL, THE FIRST FDA-APPROVED
CRISPR TREATMENT FOR SICKLE CELL DISEASE**

by

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ABSTRACT

Sickle Cell Disease (SCD) is a genetic blood disorder characterized by the production of abnormal hemoglobin, the protein in red blood cells that carries oxygen throughout the body. This abnormal hemoglobin causes red blood cells to become rigid, sticky, and shaped like sickles or crescent moons. These misshapen cells can become stuck in small blood vessels, blocking blood flow, which leads to pain, infections, acute anemic episodes, and damage to body tissues. The disease is inherited when a child receives two sickle cell genes—one from each parent. The U.S. Food and Drug Administration (FDA) approved the first ever Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) treatment for sickle cell disease. Clustered Regularly Interspaced Short Palindromic Repeats is a technology used to selectively modify the DNA of living organisms. Exa-cel, also known by the brand name Casgevy, uses cutting-edge CRISPR gene editing technology to edit the BCL11A gene, which normally stops the body from producing a type of hemoglobin that is exclusive to fetuses. By editing the DNA of BCL11A, Cas9 deactivates the protein in bone marrow stem cells, which results in red blood cells with a typical round shape and fetal hemoglobin. This novel treatment

involves removing the patient's own bone marrow stem cells, editing with exa-cel, followed by destroying the remaining, untreated bone marrow, and injecting the edited cells back into the patient. This breakthrough technique may provide patients with a long-term solution by not only addressing the underlying genetic defect and mitigating the symptoms of sickle cell disease. The study explores the progress of the treatment's advancement, from research to clinical trials for evaluating the safety and effectiveness of the treatment, Exa-cel seems to be the most current gene therapy advancement in this fast-changing technological area. An in-depth assessment of the safety record of Exa-cel is compared to an analysis of treatment options like hydroxyureas therapy and stem cell transplants to highlight the benefits of Exa-cel treatment approach over others in both the short- and long-term periods. Additionally exploring how Exa-cel affects patients' quality of life with a focus, on their reported experiences and daily life enhancements as considering issues related to accessibility and cost. Looking ahead to the possibilities of Exa-cel's role in genetic medicine innovation stands out as a breakthrough with the potential not just to revolutionize SCD care standards but also to drive progress in addressing various genetic conditions – paving the way for hopeful outcomes, for patients and the wider medical field.

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LIST OF ABBREVIATIONS

AE	Adverse Event
BCL11A	B-cell lymphoma/leukemia 11A
BMTS	Bone Marrow Transplant Subscale
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribonucleic Acid
Exa-Cel	Exagamglogene Autotemcel
FACT-G	Functional Assessment of Cancer Therapy General
FDA	Food and Drug Administration
GVHD	Graft-Versus-Host Disease
HBB	Hemoglobin Subunit Beta
HbF	Hemoglobin
HbS	Hemoglobin S
HCT	Hematocrit
HLA	Human Leukocyte Antigen
HPFH	Hereditary Persistence of Fetal Hemoglobin
HRQoL	Health-Related Quality of Life
HSCs	Hematopoietic Stem Cells
HSCT	Hematopoietic Stem Cell Transplant
HSPCs	Hematopoietic Stem and Progenitor Cells
ICER	Institute for Clinical and Economic Review
LMICs	Low- and Middle-Income Countries

Lovo-cel	Lovotibeglogene Autotemcel
PROs	Patient-Reported Outcomes
QoL	Quality of Life
RBC	Red Blood Cell
RNA	Ribonucleic Acid
RNP	Ribonuclear Protein
SAEs	Serious Adverse Events
SCD	Sickle Cell Disease
SOC	Standard of Care
TDT	Transfusion-Dependent Thalassemia
VAS	Visual Analog Scale
VOC	vaso-occlusive Crises
VOE	Vaso-occlusive Episode

INTRODUCTION

Background of SCD

A genetic condition known as sickle cell disease alters the structure and function of red blood cells in the body. It is brought on by a mutation in the gene that encodes hemoglobin, the oxygen-carrying protein found in red blood cells. Red blood cells are often spherical and flexible, which makes it easy for them to pass through blood arteries. On the other hand, aberrant hemoglobin in SCD results in stiff red blood cells that resemble crescents or sickles. These sickle-shaped cells can become lodged in blood vessels, obstructing blood flow and resulting in excruciating pain, infections, damage to organs, and strokes [Figure 1][1]. Due to the disease's autosomal recessive nature, a child cannot develop the disorder unless they inherit a copy of the faulty gene from each parent. People with African, Mediterranean, Middle Eastern, and Indian ancestry are the main groups affected. Only one copy of the defective gene is present in sickle cell trait carriers, who typically have no to extremely mild symptoms but are able to transmit the mutation on to their children.

Despite being identified in the early 1900s, the genetic and molecular causes of SCD were not understood until the mid-1900s. Advances in medical research have enabled better treatment alternatives, such as routine blood transfusions, infection prevention, and pain management. These substitutes focus on controlling symptoms and side effects. With significant morbidity and a detrimental effect on quality of life, SCD continues to be a major public health concern despite recent advancements.

Sickle-Cell Anemia

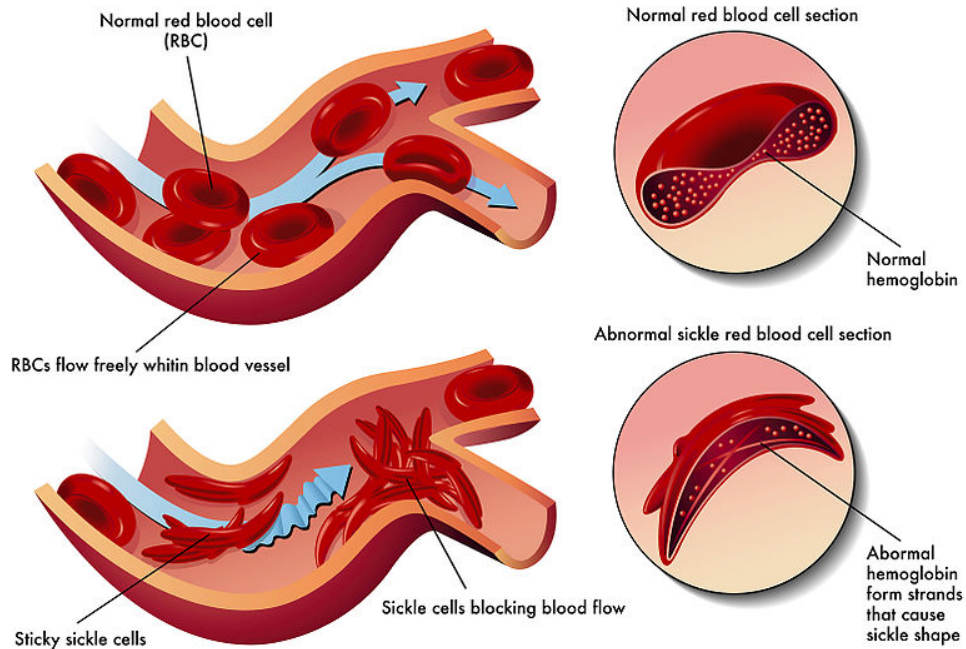


Figure 1 Risk Factor for Sickle Cell Anemia [1].

Normal hemoglobin is a protein in red blood cells (RBCs) that carries oxygen from the lungs to the tissues and organs in the body. In adult hemoglobin (HbA), it consists of four protein subunits: two alpha chains and two betas. As RBCs circulate through the body, hemoglobin binds oxygen in the lungs and releases it where it is needed. For HbA to function properly, the beta chains are extremely important. The beta chains contribute to the formation of the sites for oxygen binding and help stabilize the structure of hemoglobin so it can pick up and release oxygen efficiently. Found in the developing fetus, fetal hemoglobin (HbF) contains two gamma chains instead of beta chains. Because the gamma chains are more prone to bind to oxygen than the beta chains, the fetus can draw oxygen from the mother's blood more efficiently. Following birth, the body

gradually transitions to adult hemoglobin by switching from gamma to beta chains.

Therefore, during various phases of development, both the beta and gamma chains are essential for the appropriate transport and distribution of oxygen.

Symptoms for SCD appear as the abnormal Sickle hemoglobin (HbS) levels rise, and β -chain synthesis takes the place of γ -chain synthesis, resulting in 60% to 80% of the fetal hemoglobin at delivery [1]. After birth, 60–80% of hemoglobin is made up of HbF; during the first few months, the ratio generally decreases as the body converts to HbA. In SCD, the delayed switch from HbF to HbS may provide temporary protection, but there is a higher risk of severe side effects as HbS levels increase. Some people may reach pathological levels of HbS within 8 to 10 weeks of birth, at which point potentially deadly repercussions may ensue, however everyone experiences this transition at a different period. Dactylitis, a type of inflammation that affects your fingers and toes is one of the early issues, and the newborn may die from acute chest syndrome and abnormal splenic function, which increases the risk of excessive septicemia and acute splenic sequestration [1].

Beta-haemoglobinopathies are caused by mutations in the beta-subunit of hemoglobin, the molecule that enables red blood cells to carry oxygen. Because of a protein shortage, people with beta-thalassemia have little to no ability to carry oxygen. Anemia and long-term organ damage are caused by hemolysis, or the rupture of red blood cells, which is caused by the mutation in SCD [2]. Additionally, it results in red blood cell sickling and clumping, which can trigger excruciating inflammatory vaso-occlusive crises. Currently

available small-molecule drugs for SCD reduce pain, morbidity, and mortality but do not treat the illness.

The Need for Innovative Treatment

The major therapeutic approaches to SCD have so far remained blood transfusions and hydroxyurea. The FDA approved hydroxyurea for use in adults in 1998, while blood transfusions were first used in the 1970s to help SCD patients have fewer recurring strokes [3]. Conventional SCD treatments utilize the medication hydroxyurea also known as hydroxycarbamide, to assist in pain reduction and to prevent complications. Blood transfusions are considered to raise the number of healthy red blood cells. A bone marrow and blood transplant has been the sole treatment for SCD for the longest time, although this is not always the case. Many sufferers of SCD may not have a genetically related relative who could donate.

A donor must be appropriately matched to provide a patient the best chance of a successful transplant. Approximately 85% of children who get bone marrow and blood transplants are successful when the donor is related and their human leukocyte antigen (HLA) matches [4]. Regardless of their very high success rate, transplants remain somewhat risky. Complications include seizures, serious infections, and other health problems. A total of about 5% of those who receive these transplants die. Sometimes, cells from the transplant may cause damage to organs of the person receiving them; this

is called graft-versus-host disease. Although medication is available to help, the probability of this occurring is still great.

Many of the treatments, for SCD come with side effects and are often ineffective in addressing the condition effectively. Due to this limitation in treatment options research is actively exploring strategies. Recent studies have been focusing on stem cell therapy to repair damaged cells and improve bone marrow function. Another promising avenue is gene therapy, which aims to address the root cause of the disease. One notable advancement, in this area is a treatment known as exagamglogene autotemcel called Exa-cel. Individuals affected by this disorder are now filled with optimism thanks to a groundbreaking gene therapy recently authorized by the FDA. Exa-cel treats the underlying genetic defect that causes SCD, whereas standard medicines primarily concentrate on managing symptoms. Throughout the therapy, the patient's own hematopoietic stem cells are altered to create large quantities of fetal hemoglobin, which is naturally found in newborns and has the ability to stop red blood cells from sickling.

Overview of Exa-cel Therapy

Using autologous, ex vivo CRISPR-Cas9 therapy, the patient's blood stem cells are modified to create high quantities of fetal hemoglobin in red blood cells, a process known as Exa-cel. During fetal development, the oxygen-carrying HbF is naturally present, but it often shuts down after delivery in part to the expression of BCL11A. Treatments for SCD have included genetic editing techniques such as CRISPR-Cas9 to boost the expression of fetal hemoglobin by disrupting its suppression **[Figure 2][5]**.

Exa-cel uses a CRISPR–Cas9 nuclease that precisely cuts DNA to mute BCL11A, a repressor of the HbF gene, to modify hematopoietic stem and progenitor cells extracted from the patient's bone marrow and changed ex vivo, or outside the body. Using a method similar to blood donation, the patient's own hematopoietic stem cells are extracted as the initial stage in the treatment procedure.

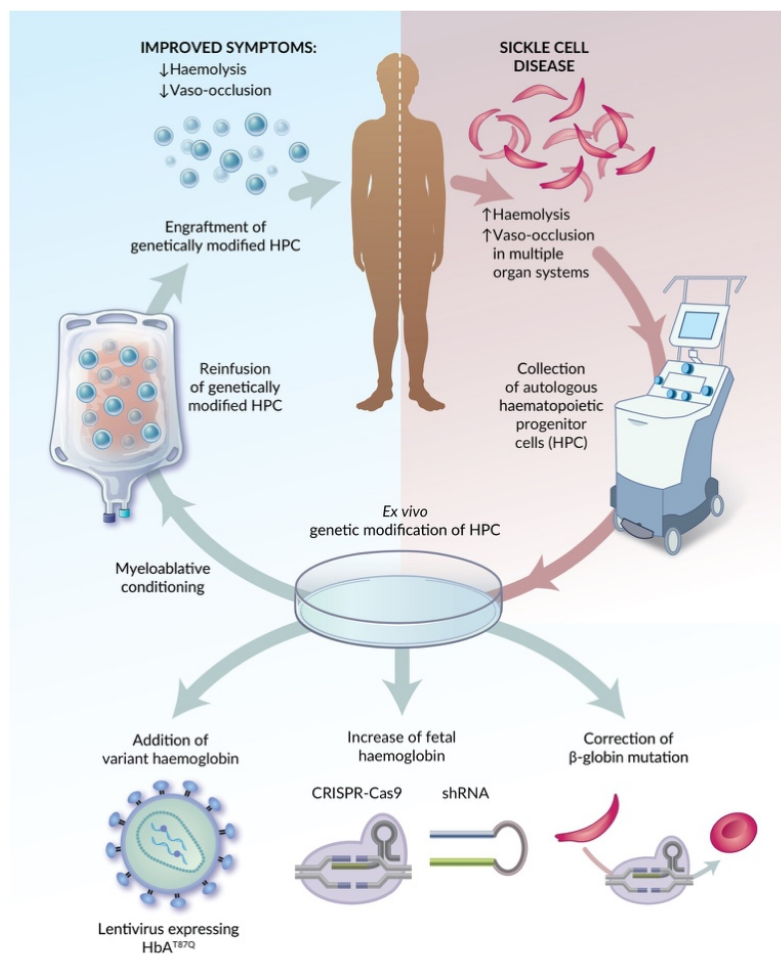


Figure 2 Key Stages in the Process of Gene Therapy [5].

These cells are then carefully altered in a laboratory setting. In order to allow the newly altered cells to engraft, the patient undergoes a conditioning treatment that includes chemotherapy after the cells have been genetically modified. The mutated cells proceed to the bone marrow after being reintroduced into the patient's circulation, where they begin to generate new, healthier blood cells with elevated expression of fetal hemoglobin.

I. UNDERSTANDING EXA-CEL THERAPY

Mechanism of Action

The novel gene-editing treatment Exa-Cel, aims to address the underlying genetic etiology of SCD. A mutation in the β -globin gene causes SCD by producing hemoglobin S, an abnormal form of hemoglobin that polymerizes in low oxygen tension environments. RBCs undergo sickle-shaped deformation as a result of this polymerization, which can impede blood flow and lead to serious side effects include hemolytic anemia, chronic pain, vaso-occlusive crises, and other end-organ dysfunctions [6]. Exa-Cel uses the CRISPR-Cas9 gene editing technique to specifically target and interfere with the expression of the BCL11A gene in hematopoietic stem cells. A transcriptional repressor called BCL11A is essential for postnatally suppressing the production of fetal hemoglobin.

The bulk of HbA is produced by healthy people, whereas HbF production is stopped. However, high levels of HbF are linked to a milder clinical pattern in SCD patients because HbF does not participate in the pathological sickling process [6]. By inhibiting the BCL11A enhancer through targeted CRISPR editing, Exa-Cel aims to reactivate HbF synthesis and undo the negative consequences of HbS. There is a strong case for moving Exa-Cel along the development pipeline because preclinical research and early-stage clinical trials have shown that altering the BCL11A enhancer can significantly raise HbF levels in edited cells [6]. **Figure 3**[7] shows both the pathological and genetic mechanisms for SCD. Mutations in the HBB gene lead to low oxygen conditions which then result in sickle shaped RBCs. This in turn causes vaso-occlusion and further contributed to the side effects linked to SCD. The mutation in the HBB gene is caused by the replacement of glutamic acid with valine in hemoglobin. Exa-Cel seeks to reverse the issue by working to reactivate the fetal hemoglobin production.

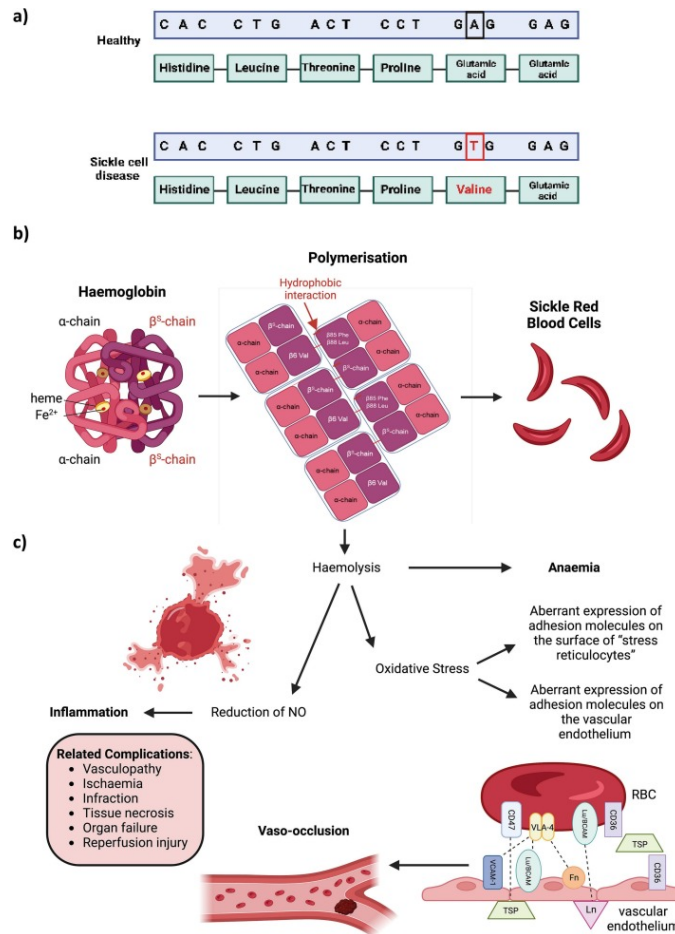


Figure 3 Schematic representation of Exa-Cel therapy mechanism of action [7].

The precision of CRISPR-Cas9 allows targeted changes with few collaterals off-target effects, which is an important consideration for gene therapy. Guided by a specific RNA sequence, the Cas9 enzyme induces a double strand break at the BCL11A enhancer, which the cell attempts to repair, often generating a deletion that disables BCL11A expression. Besides increasing the production of HbF, this established process maintains

overall genomic integrity of the HSCs for the assurance of safety and efficacy of the therapy. Long-term stable expression of HbF in patients receiving Exa-Cel is an important development in the treatment of SCD.

Development and Design

Early studies of Exa-Cel concentrated on the relationship between HbF levels and the severity of SCD, which led scientists to speculate that increasing HbF production would have therapeutic advantages. Gene editing was investigated as a potential treatment after observational studies of people with hereditary persistence of fetal hemoglobin revealed that higher HbF levels were linked to a noticeably milder phenotype of SCD [8]. Exa-Cel was specifically designed to apply CRISPR technology to the target BCL11A enhancer for enabling selective induction of HbF in HSCs. This procedure requires precision when modifying the genetic elements controlling the switch in hemoglobin production from fetal to adult life. To do this without disrupting the normal expression of the β -globin gene itself, scientists engineered a CRISPR-Cas9 technology that selectively targets the BCL11A enhancer and works to shut down the inhibitory effect of BCL11A on HbF production. The creation of Ex Cel involved a lot of trial and error as researchers carefully aimed to modify the BCL11A enhancer without affecting any other genes in the process.

An important benefit over in vivo techniques is the therapeutic design's use of an ex vivo editing approach, which enables careful quality control throughout the gene-editing procedure. Ex vivo HSCs are extracted from the patient, modified to interfere with the

BCL11A enhancer, and then reintroduced into the patient's circulation. This procedure lowers the possibility of immunological reactions that can arise from introducing foreign substances into the patient and also makes it easier to closely monitor the editing process since it occurs outside of the patient's body.[8]. Additionally, by concentrating on the BCL11A enhancer, a tailored strategy that reduces the possibility of interfering with other vital cellular processes is achievable. Exa-Cel uses sophisticated guide RNA design and CRISPR system optimization for selectivity in order to produce large amounts of HbF with little off-target activity. Precision medicine, which tailor's treatments to each patient's unique genetic composition to increase efficacy while lowering risks, is embodied in this design philosophy.

The CRISPR-Cas9 technology used in Exa-Cel treatment is further explained via the schematic representation in **Figure 4[9]**, which emphasizes the technique's accuracy and design. The diagram shows how CRISPR/Cas9 genome editing uses a single guide RNA to lead the Cas9 enzyme into the specific region of DNA allowing it to create a double-strand break. With the help of the donor DNA, the cell can introduce the new genetic material. In the case that a donor DNA is not available, the cell then repairs the break via natural mechanisms which often result in genetic mutations such as deletions or insertions, disrupting the gene's natural functions [9]. CRISPR/Cas9 is maintained throughout the activity of two key components that make up the system: a guide RNA and an effector enzyme which is typically Cas9 that comes from the type II CRISPR/Cas system of *Streptococcus pyogenes* as illustrated in **Figure 4[9]**.

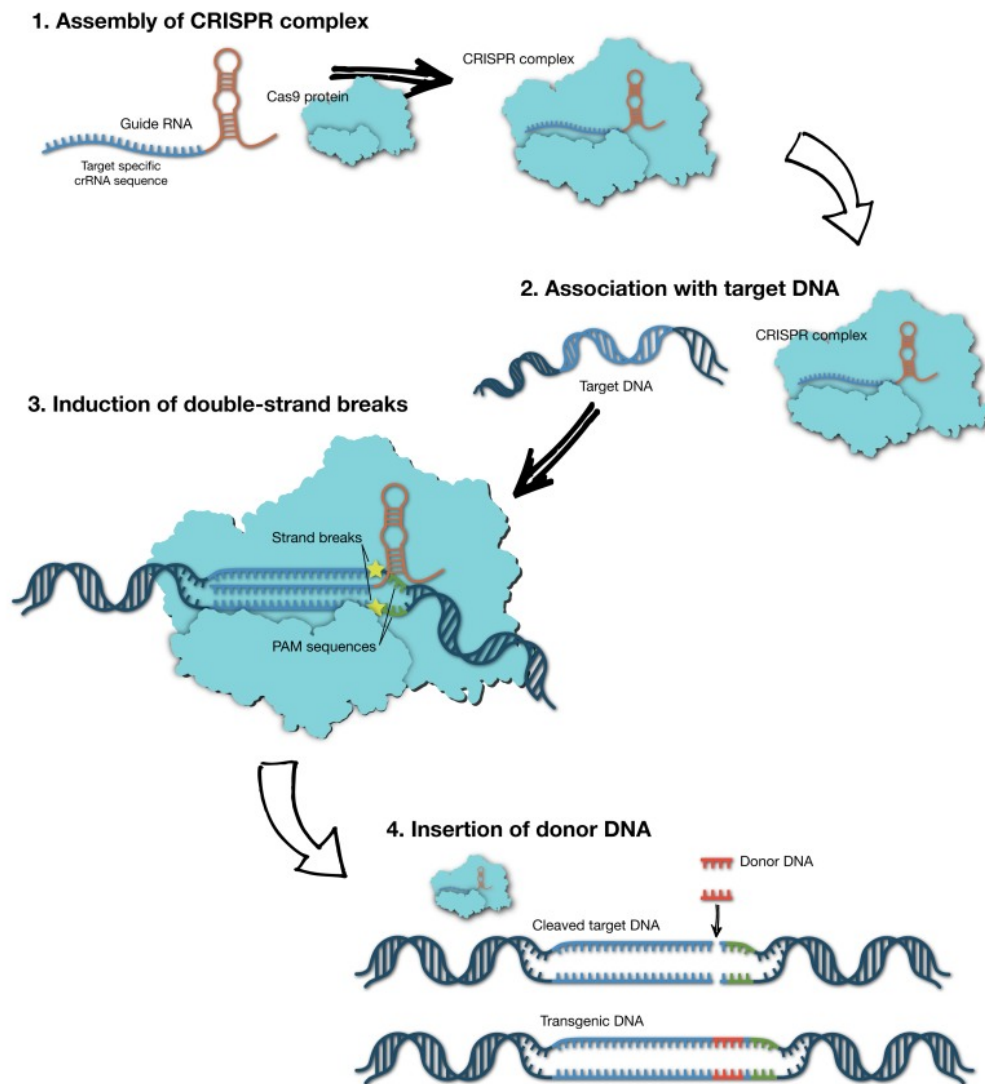


Figure 4: CRISPR-Cas9 schematic representation relevant to Exa-Cel therapy [9].

The integration of these advanced technologies has transformed Exa-Cel into a viable treatment for patients with SCD and is the beginning of further successes in gene therapy. Researchers, physicians, and industry partners have come together to create a drug that

not only addresses the underlying genetic cause of SCD but will also have the potential to revolutionize the approach to SCD worldwide.

Preclinical Studies

Before Exa-Cel was used in clinical settings, preclinical research was essential in confirming its effectiveness and safety. Early studies evaluated the biological effects of blocking the BCL11A enhancer and simulated human illness states using mouse models. These investigations showed that CRISPR-mediated editing successfully raised HbF levels, which were directly associated with a decrease in red blood cell sickling. Exa-Cel was advanced to human trials for a strong reason: the altered cells' increased capacity to make HbF [10].

Human-derived HSCs were used in the study in addition to mouse models, which further supports the findings' therapeutic significance. In vitro research revealed that HSCs with the BCL11A enhancer disabled produced significantly more HbF than cells without the edit. These outcomes demonstrated Exa-Cel's potential to revolutionize the therapy landscape for people with SCD in addition to validating the focused mechanism of action [10]. Given the ramifications of gene editing, safety assessments were an essential aspect of the preclinical stage. Using next-generation sequencing technologies, a thorough off-target study was carried out to make sure the CRISPR-Cas9 system only affected the designated BCL11A enhancer area. Addressing safety concerns and preventing unexpected genetic modifications—which could potentially result in negative consequences or oncogenesis—were made possible by this comprehensive evaluation.

Preclinical research also investigated how well the altered cells stood the test of time. Indeed, the edited HSCs increased HbF production not just immediately after the gene editing but continued to express the higher amount over an extended period of time. It indicates the Exa-Cel benefit could be long-lasting-a key consideration when reviewing the success of gene therapies over the longer term. This is especially important for chronic conditions, like SCD, which require ongoing treatment often throughout an individual's lifetime.

II. CLINICAL TRIALS AND EFFICACY

Safety and Tolerability

A cure for SCD is still urgently needed, despite the fact that several treatments have been produced over the years. Exagamglogene autotemcel, a non-viral CRISPR-Cas9 gene-editing therapeutic, is one of the most intriguing advancements in this area. By altering autologous hematopoietic stem and progenitor cells to restore the production of fetal hemoglobin, Exa-cel aims to address the underlying etiology of SCD.

An ongoing phase 3 clinical investigation called the CLIMB SCD-121 trial is investigating the effects of Exa-Cel in patients with severe SCD who are between the ages of 12 and 35 [11]. The treatment showed eliminated vaso-occlusive crises in 97% of the participant patients with SCD over a 12 month or more period. Exa-Cel has shown considerable therapeutic promise in several regions, according to the trial's encouraging

results as of February 2023. Remarkably, 95% of trial participants achieved the main goal of being free of harmful volatile organic compounds for at least a year. Furthermore, all patients (100%) had not been hospitalized for VOCs for a minimum of 12 months in a row. Given that VOCs are among the most common and incapacitating side effects of SCD, these findings indicate a significant improvement in patient outcomes. The extended duration of VOC-free status in these patients following therapy raises the possibility that Exa-Cel may have long-term effects and provide a workable remedy for those with the worst illness symptoms. The duration of VOC independence ranged from 12.3 to 41.4 months, and 29 out of 30 patients (96.7%) were free of VOCs for at least 9 months [11].

Fetal hemoglobin levels were also shown to have significantly increased during the trial. Exa-Cel reduces sickling and its related problems by promoting the generation of normal-shaped red blood cells by reactivating fetal hemoglobin production through gene-editing of the BCL11A gene. Patients had a mean HbF level of 36% of total hemoglobin at month 3 following infusion, and levels were often kept above 40% starting in Month 6 [11]. The fact that more than 95% of red blood cells express HbF and that this distribution is pan cellular further indicates that the therapy was successful in producing the desired result.

In the CLIMB SCD-121 trial, the Exa-Cel treatment showed a largely positive safety profile. Although every patient in the trial had at least one adverse event (AE), most of

them were mild, with most being grade 1 or 2 in severity. This suggests that the therapy was generally well tolerated. The most frequent AEs were headache (52.4%), febrile neutropenia (52.4%), stomatitis (61.9%), and nausea (66.7%), all of which are major side effects linked to myeloablative conditioning needed for stem cell therapy [11]. Before the Exa-Cel infusion, patients in the trial underwent myeloablative conditioning with pharmacokinetically dose-adjusted busulfan, which is a treatment process used to prepare a patient's bone marrow for hematopoietic stem cell transplant. The side effects from the trial did not indicate any novel or unexpected toxicities related to Exa-Cel but were expected for this type of stem cell transplantation. Most of the grade 3 or 4 AEs, occurring in 95.2% of patients, happened in the first six months post-infusion. However, none of these serious AEs were related to medication Exa-Cel. There were no tumors or graft failures reported from the trial, which is critical for any treatment that involves gene editing. Even as the rate of major side events was higher than for conventional treatment procedures, the lack of direct causality with Exa-Cel suggests that the therapy itself does not carry new, intolerable risks. The trial documented that a total of 20 patients (45%) had serious adverse events, none of which were considered to be related to the Exa-Cel therapy according to the investigators. Only one patient (2%) died from COVID-19-related respiratory failure, which also was unrelated to do with the Exa-Cel therapy itself [11]. This emphasizes how crucial it is to take outside variables like viral diseases into account when evaluating the general safety of gene-editing therapies. It was also noted that no participants left the study because of side effects.

Efficacy Assessment

Exa-Cel targets and damages the BCL11A gene using CRISPR-Cas9 gene-editing technology, which reactivates the production of fetal hemoglobin. This change helps decrease the development of sickled cells while at the same time decreasing the disease's potentially fatal side effects. The strong data from clinical studies supporting the therapy's effectiveness and its implications for the management of SCD is covered in this section.

In critical clinical trials, Exa-Cel had impressive effectiveness in lowering the frequency and intensity of vaso-occlusive crises, one of the most incapacitating side effects of SCD. Patients with severe illness receiving Exa-Cel therapy reported significant therapeutic benefits. One such Phase 1/2 trial had almost 90% of individuals report complete halting of VOCs over a follow-up period of more than two years, thus showcasing the potential of this therapy for long-lasting illness change [12]. Curiously, trials also found that median HbF levels attained by patients' post-therapy were sufficient to significantly reduce cellular sickling and hemoglobin polymerization-representing the underlying pathophysiological mechanisms behind the disease.

In addition to lowering VOCs, Exa-Cel demonstrated potential in lowering patients' reliance on ongoing blood transfusions. Exa-Cel demonstrated its revolutionary therapeutic potential by enabling transfusion independence for a subset of patients with transfusion-dependent SCD within months of therapy [13]. The effects of the medication on fetal hemoglobin levels and the removal of transfusion needs are visibly shown in

clinical trial data [Figure 5][13]. Accordingly, the studies also significantly improved important biomarkers of hemolysis, including reticulocyte counts and lactate dehydrogenase levels, indicating a reduction in chronic anemia, which is characteristic of SCD.

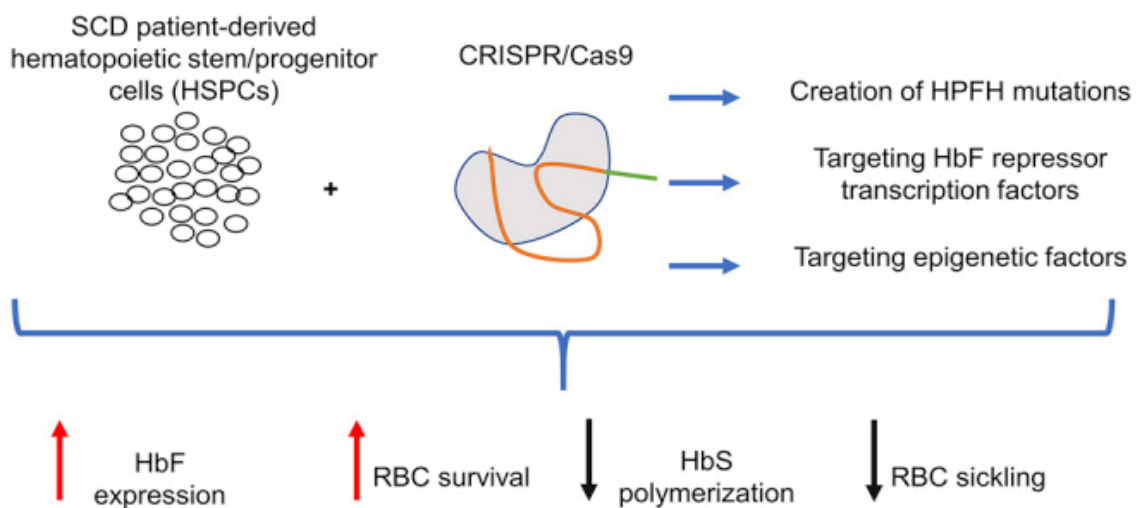


Figure 5 Fetal Hemoglobin Increases Following Exa-Cel Therapy in Sickle Cell Disease Patients. Reprinted from New England Journal of Medicine, Frangoul et al., 2021, under the CC BY-NC-ND license [13].

These clinical outcomes are supported by improved quality of life. Since Exa-Cel treated both the clinical burden and the psychological distress impinged by severe SCD, patients often reported improved mental health and reduced physical limitations. Exa-Cel 's ability to alter the genetic underpinnings of the disease represents a paradigm shift in the

treatment of the disease, as previous treatments focused on the mitigation of its symptoms.

The application of CRISPR-Cas9 technology in the usage of Exa-Cel promised far greater accuracy and efficiency in the performance of gene editing than any other genetic therapies. In contrast, while effective, such lentiviral-based modalities as lovo-cel are less precise than CRISPR-mediated modalities in targeting specific genomic loci [14]. These advances do, nonetheless, entail some question marks. Given that the clinical data to date extend only several years of follow-up, it is not yet evident how durable the effects of Exa-Cel will prove to be.

Cost factors should also be taken into account. The creation of Exa-Cel is a significant advancement in customized medicine, but it also raises concerns about equity and accessibility. Given the demographics of SCD prevalence, it is concerning that underprivileged communities may be disproportionately impacted by the high price of modern gene-editing therapy. These monetary obstacles emphasize how crucial it is to create laws that guarantee fair access without sacrificing sustainability. Exa-Cel 's clinical effectiveness highlights its potential to transform the treatment of SCD in spite of these obstacles. The treatment is positioned as a viable therapeutic strategy due to its capacity to restore normal hematological function and eradicate potentially fatal consequences. In order to validate these advantages over an extended period of time and investigate the

therapy's application in broader populations, including young patients, ongoing Phase 3 trials and post-approval research will be essential.

Exa-Cel 's effectiveness makes it a noteworthy development in genetic medicine. Clinical study results show that it can address the underlying cause of SCD, providing a transformational approach that goes beyond symptom management. Although there are still issues with durability, cost, and long-term safety, the treatment offers patients hope and serves as a model for further genetic therapies. Realizing its full potential and making sure that everyone may benefit from it will require ongoing research and innovation.

Ongoing Research and Future Directions

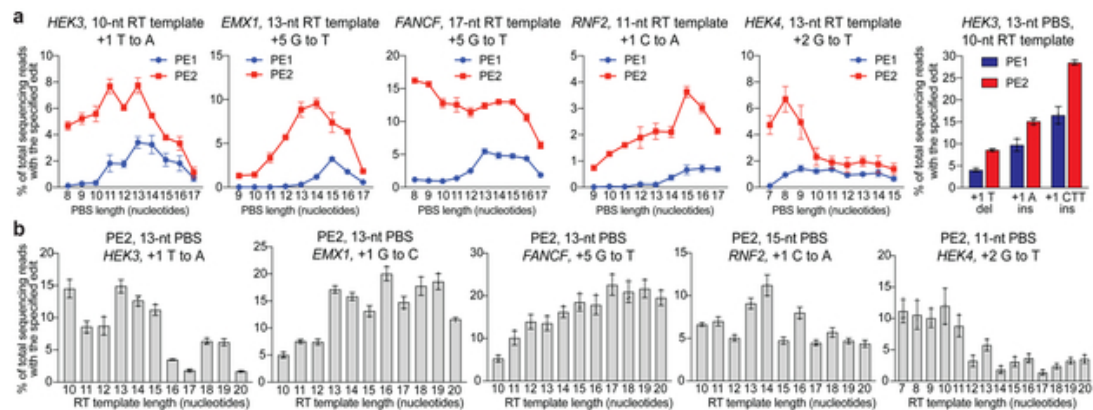
While the breakthrough of Exa-Cel stands as a possible cure for treating SCD, further research is still needed to maximize the treatment's effectiveness, improve the overall safety of the mechanism, and broaden access of the treatment to a larger segment of the population. Continuing research aim to expand the applications of gene editing technique for other disorders, such as beta-thalassemia, Huntington's disease, and Duchenne muscular dystrophy that are genetically stemmed. One of the more significant areas of current ongoing gene therapy research is ensuring the long-term effectiveness of the Exa-Cel. Reduced incidence of vaso-occlusive crises and transfusion independence are the results of promising early clinical trials. Researchers are still working to determine if fetal hemoglobin reactivation endures throughout time, despite some encouraging results. More research is required to determine whether these early outcomes

are long-term sustainable, as concerns about long-term organ damage and the emergence of other problems later in life remain. Making sure that the advantages of Exa-Cel last a patient's entire life is crucial to making this treatment a practical choice [15].

With technological advancements in the development of gene-editing tools come new modalities, such as base and prime editing, which can probably adjust more accurately than CRISPR-Cas9, promising novel avenues. Base editing, which converts one DNA base insert to another at a position with minimal risk of double strand breaks of DNA, generally indentured with unintentional alterations, will serve to allow gene editing therapies with more accurate target action and fewer side effects. In this respect, it has also been claimed that prime editing may provide more safety against CRISPR methods, as it uses no double-strand breaks for its mode of action in manipulating DNA sequences, making it safer [16]. In fact, these technologies could lend themselves to an array of applicable diseases and hence making genetic therapy for SCD safer and more efficacious.

The diagram underneath [Figure 6][16] shows the enhancement of prime editing ability applying the developed prime editor 2 (PE2) system. Panel (a) depicts the manner in which changes are made on the reverse transcriptase enzyme to reinforce the editing tools that can be applied in different locations of the genome of HEK293T cells and the minor insertions, deletions, and a very particular location in HEK3 cells. The impact of reverse transcriptase template length on editing efficiency was shown in Panel (b), and this gives

rise to the possibility of exactly trimming genomes. Discovering the effectiveness of prime editor 1 (PE1) in the occurrence of point mutations, targeted insertions, and deletions at various genomics without the use of donor DNA templates, in other words, double-strand breaks will, furthermore, be evinced by the findings coming forth. For instance, PE1 at one genomic locus, could incorporate and delete with efficiencies of 4-17%, thereby displaying its capability of performing transversions, insertions, and deletions with little formation [16]. PE1 uses a standard reverse transcriptase and has lower editing efficiency, requiring longer sequences to work effectively. PE2 improves on PE1 by using a modified reverse transcriptase with mutations that make it faster, more accurate, and able to work with shorter sequences, leading to 1.6- to 5.1-fold better editing efficiency as illustrated in [Figure 6][16].



In addition to technology improvement, scientists focus on the other two pressing *Figure 6 Prime editing of genomic DNA in human cells by PE1 and PE2 [16].*

aspects: making the treatments cheaper and more readily available. Myeloablative conditioning, a preparatory procedure in which the patient's bone marrow is removed to

make room for the modified cells to engraft and expand, is currently the most significant impediment. In hopes to reduce treatment risks and costs, other possible alternatives, such as antibodies, are under study. The idea is that simplification of the delivery procedures might lead to a broader use of Exa-Cel and related medications, particularly in the regions where SCD is common, i.e., in the places with low resources [15]. To bring these treatments to a global population, especially where SCD is prevalent, lowering the price and making these treatments more available are crucial steps.

Combination therapy is also another thrilling area where humans can explore possibilities. Although Exa-Cel facilitates SCD's genetic correction, these complimentary therapies might make the resultant outcomes maximum. Medications that stimulate the production of fetal hemoglobin, the form of hemoglobin mainly found in newborns up to eight weeks of age, or gene-editing therapy are possible approaches [15].

One of the top priorities for researchers is to ensure Exa-Cel is safe for the long term. The possible concerns, like insertional mutagenesis and off-target consequences, need to be closely monitored despite the fact that the short-term safety profile seems favorable. Addressing these issues and guaranteeing the ongoing effectiveness of gene-editing treatments would need the creation of improved bioinformatics tools, improved delivery systems, and strict safety monitoring procedures. To make sure that these cutting-edge treatments continue to be safe and successful over time, long-term patient follow-up is required [16].

III. SAFETY PROFILE AND ADVERSE EVENTS

Short-Term Safety

Gaining more information about the short-term safety of Exa-Cel is significant to determine the treatment's clinical feasibility, especially in a disease as complex as SCD, whose rates of morbidity and comorbidities are already high. Besides the risk of targeted editing, the conditioning regimen, immunological reactions, and immediate health effects on the patients after the treatment are also to be factored in when it comes to short-term safety.

The most commonly cited adverse events in the Exa-Cel clinical trials, so far, have been related to the conditioning drug treatment that precedes the administration of the modified cells. The latter typically includes chemotherapy or other myeloablative drugs that are meant to ablate the bone marrow of the patient. This is a necessary step to create space for the administration of the gene-modified hematopoietic stem cells. Although conditioning is a common practice in stem cell-based treatments, there are risks associated with it. The risks include infections, neutropenia, or a low white blood cell count, and other systemic issues. These side effects are common in myeloablative-conditioned gene therapies and are not unique to Exa-Cel.

A study was done in 2016 that used CD34+ HSPCs from patients with SCD to test their modifications and engraftments in immunodeficient mice. The study involved multiple HSPC samples derived from patients for ex-vivo testing and evaluations in mice [17]. Overall, most AEs observed in the phase 1 and 2 trials of Exa-Cel were graded as mild to moderate. In general, the severity of AE related to the conditioning regimen was low. Serious adverse events, including potentially fatal infections or severe neutropenia, were rare, and when they occurred, they were treated appropriately with standard medical interventions, including administration of antibiotics and growth hormones to stimulate the production of white blood cells. The Exa-Cel study showed minimized risks for neutropenia, a complication commonly linked to myeloablative conditioning [17]. The study showed successful editing of the HBB gene in CD34+ HSPCs (which encodes the beta globin protein) using Cas9 ribonucleoproteins to achieve correction of the sickle cell mutation. Improvement in the hematological parameters of patients during the conditioning regimen was remarkable since signs of effective engraftment and cellular healing were obtained. Such rapid recovery could indicate low chances of severe, long-lasting side effects of the treatment.

Apart from the training regimen, the potential of unwanted effects of the CRISPR-Cas9 gene editing technique itself opens the floor for more short-term safety questions of Exa-Cel. The fact that CRISPR-based treatments have off-target effects is generally recognized, meaning that other regions of the genome could become unintentionally edited with negative outcomes such as the formation of cancer or other deleterious

mutations. This has been countered by the introduction of inclusive off-target effect monitoring, including whole-genome sequencing and other methods in the clinical trials of Exa-Cel to detect unexpected genetic changes. Clinical experiments on Exa-Cel have demonstrated that there was little chance of off-target effects and no indication of clinically significant alterations that could have negative consequences. A notable limitation highlighted was the difficulty of targeting HSCs in clinically relevant contexts, though the method is favored to overcome challenges in efficient engraftment [17]. These results suggest that although off-target effects are still a theoretical issue, it is unlikely to present a serious risk in the near future.

In order to better comprehend how Exa-Cel reduces the possibility of off-target effects, [Figure 7][17] shows how human hematopoietic stem and progenitor cells precisely edit the HBB locus, which is the location on chromosome 11 that codes for beta hemoglobin. This diagram illustrates how the CRISPR/Cas9 system can produce site-specific double-strand breaks at the β -globin gene's location, the HBB locus. The editing process is highly targeted with few off-target alterations when recombinant adeno-associated virus is used as a donor template for homologous recombination. The image demonstrates the exact editing mechanism's phases in detail and emphasizes how CRISPR/Cas9 and rAAV6 work together to enable accurate and efficient genome modification, which is necessary to achieve the targeted therapeutic result without inadvertently harming others.

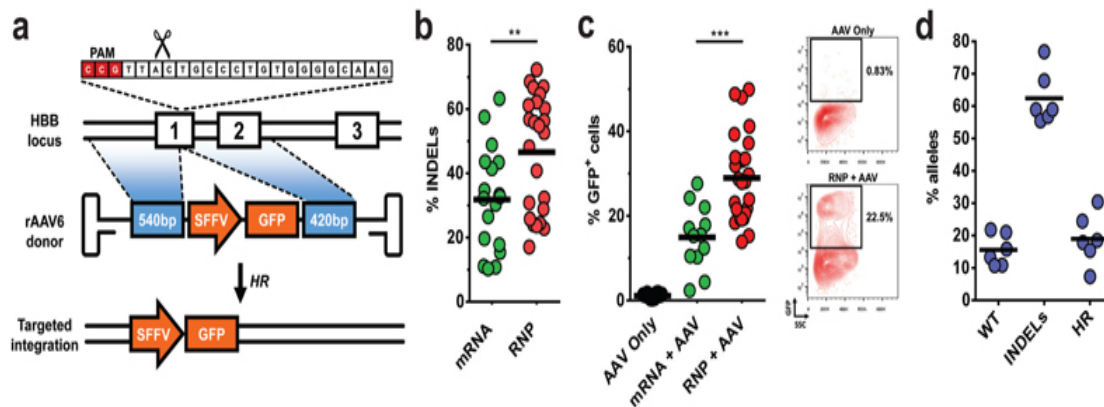


Figure 7 CRISPR/Cas9 and rAAV6-mediated targeted integration at the HBB locus in human CD34+ hematopoietic stem and progenitor cells (HSPCs) [17].

Another critical feature of the short-term safety profile of Exa-Cel is insertional mutagenesis. The process by which altered genetic material inserts itself into the genome in an unintended location can lead to insertional mutagenesis, which may interfere with normal gene expression or turn on oncogenes, both of which increase the risk of cancer. This is a complication well understood with gene therapies that insert new genetic material into the host genome. However, studies related to Exa-Cel have not documented any definitive proof of insertional mutagenesis or the risks associated therewith in the short-term follow-up. Supportive evidence of the fact that the treatment does not hold any increased risk for insertional mutagenesis was provided by a study conducted in 2023 [18]. The study, CLIMB SCD-121 is a phase 3 experimental study analyzing the effects from a single dose of Exa-Cel seen in patients between the ages of 12-35 years old with SCD, who also experience frequent vaso-occlusive crises. With data from 17 adults who have been monitored over the course of 16 months post-infusion (as of February 10,

2023), the trial demonstrates changes observed in patient-reported outcomes over the period of two years.

The short-term safety profile of Exa-Cel takes into account not only the gene-editing process but also the patients' general recuperation after the gene therapy. Patients usually have a brief period of myelosuppression following the infusion of the modified stem cells, during which the bone marrow's capacity to generate blood cells is inhibited. This is a typical aspect of the recuperation process after stem cell-based treatments and is anticipated after the conditioning regimen. In addition to symptoms including weariness, an elevated risk of infections, and a propensity to bruise or bleed more readily, myelosuppression can cause a transient drop in red blood cell, white blood cell, and platelet counts. The majority of patients experience a recovery in blood cell counts as the modified stem cells engrafts and start generating healthy blood cells, although this benefit is just temporary. Notably, the recovery was quick, and patients exhibited notable clinical improvement at the end of their initial recovery phase, including a decrease in symptoms associated with SCD, such as vaso-occlusive crises. By month 24, the Functional Assessment of Cancer Therapy General (FACT-G) total score went up with improvements observed in all 4 subscales: physical, social/family, emotional, and functional well-being [18]. These improvements were noticed as early as six months following therapy and persisted across the 24-month follow-up period. Significantly fewer vaso-occlusive crises occurred after treatment, which is consistent with patients reporting less discomfort and better general health. These findings not only point to the

possible long-term advantages of Exa-Cel, but they also imply that the therapy's short-term safety profile is linked to early clinical improvements in patient health, which could lessen the therapy's initial hazards.

Exa-Cel shows a good safety profile for the treatment of SCD in the short term, and its short-term effects, during gene-pictore selection and conditioning regimen, are generally transient and manageable. Although no serious long-term consequences were recorded, it was noted that a number of adverse events, including infections and neutropenia, typically presented with mild cases treated medically. For patients with shorter underlying disease periods, no danger signal was present during this brief follow-up time, and the risk of off-target consequences or insertional mutagenesis seemed very low. In addition, patients' newly found health-related quality-of-life gains signal short-term beneficial effects of Exa-Cel, namely, a manageable safety profile. Current data indicates that Exa-Cel has short-term safety and potential for treating SCD that holds promises as a revolutionary cure; however, extended follow-up periods, and continuous monitoring will be required.

Long-Term Safety

As the new CRISPR treatment progresses towards application ensuring the sustained safety of exagamglogene autotemcel remains a key focus of concern. The durability and safety of Exa-Cel, in maintaining effectiveness without harmful impacts is a critical aspect under scrutiny in the extensive evaluation of this therapy's long-term effects. Through analysis and real-world assessments valuable insights have been gained

on the treatments endurance and safety profile offering prospects for its use, in managing SCD.

According to preliminary findings from clinical trials, Exa-Cel has a favorable safety profile for SCD patients. According to statistics from The Lancet, 29 out of 30 patients treated with Exa-Cel experienced no vaso-occlusive crises for at least a year, with a median follow-up of 19 months. Crucially, no unanticipated toxicities or secondary cancers were documented throughout this time, highlighting the treatment's short-to mid-term safety. However, worries about possible long-term issues, like recurrent cancers, continue to exist and highlight the need for prolonged monitoring. For instance, the significance of continuous monitoring in gene-editing treatments has been illustrated by cases of acute myeloid leukemia after gene therapy in analogous situations, such as lovo**t**ibeglogene autotemcel [19].

Beyond lowering VOCs, Exa-Cel has the potential to improve overall survival and lessen problems associated with illness. The treatment was expected to extend life expectancy by 17.8–20.5 years compared to standard care (SOC) in a comparative Markov cohort model. Due to Exa-Cel 's therapeutic properties, this model also shown notable decreases in long-term consequences, including osteoporosis, diabetes, and hypogonadism. The likelihood of hypogonadism, for instance, dropped from 69.1% under SOC to 9.7–12.0% with Exa-Cel, and comparable declines were observed for osteoporosis and diabetes.

Because Exa-Cel addresses both clinical and quality-of-life aspects, these results further indicate its potential to revolutionize the long-term therapy of SCD [20].

Exa-Cel 's long-term safety depends on ongoing monitoring for late-onset effects, despite the encouraging clinical and model-based results. Although the off-target effects of gene-editing treatments like Exa-Cel are theoretically rare in clinical trials, they have the potential to eventually cause genomic instability or cancer. The prevention of major off-target alterations during Exa-Cel administration has been made possible in large part by whole-genome sequencing and strict monitoring. Long-term data gathering is essential for assessing the therapy's durability and verifying the lack of late-emerging toxicity, according to the Lancet study [19]. Beyond the safety of individual patients, a larger issue is the availability of gene-editing treatments like Exa-Cel, especially in low- and middle-income nations (LMICs), where the majority of SCD patients live. Exa-Cel has potential in high-income environments, but its broad use may be limited by its expense, infrastructural needs, and demand for extremely specialized care. When taking into account societal considerations like decreased caregiver load and increased production, cost-effectiveness assessments indicate that Exa-Cel, which costs about \$2 million, could be justified [19]. To make these cutting-edge treatments available everywhere, however, more scalable, and reasonably priced solutions are required.

The review of the Markov cohort model identifies gene-editing techniques as playing a crucial role in reducing health-care costs. Exa-Cel improves benefits to patients and

reduces encumbrances to healthcare systems by preventing problems in their tracks, thus eliminating reliance on lifetime red blood cell infusions [20]. Whether this gene therapy will be successful depends on how well it is integrated into broader health systems and also on long-term studies to confirm safety and efficacy in diverse patient populations. Exa-Cel boasts a quite impressive long-term safety profile, as initial clinical and model-based findings are highly suggestive of a considerable reduction of disease-related complications, improved survival rate, and the appearance of manageable adverse effects. Long-term monitoring, however, cannot be overestimated, and the effects of this therapy need to be studied over a longer period. Exa-Cel treatment-like interventions promise revolutionary treatment for SCDs, but this can only be a reality with further development of gene-editing technology and health infrastructure, especially in LMICs.

Management of Adverse Events

Results of the use of exagamglogene autotemcel, a CRISPR-Cas9-based gene therapy, in the treatment of SCD are promising. However, its application opens up questions about the side effects of this approach, similar to any new therapeutic approach. Understanding the side effects associated with its use is important in judging its safety profile, especially in view of the clinical studies in SCD. It is useful to understand the main characteristics and therapeutic profile of Exa-Cel before reviewing in detail its safety profile and adverse effects [Table 1][21].

Drug name (generic)	exagamglogene autotemcel
Phase (for indication under discussion)	registered
Indication (specific to discussion)	sickle cell disease and transfusion-dependent beta thalassemia
Pharmacology description/mechanism of action	Genome editing cellular therapy - induces a target double-strand break to disrupt a GATA1 binding site in the erythroid specific enhancer of the BCL11A gene by non-homologous end-joining repair, which leads to silencing of this gene and subsequently to the production of HbF
Route of administration	Injectable, intravenous
Pivotal trial(s)	CLIMB THAL-111 [20] CLIMB SCD-121 [23]

Table 1 Drug Summary Box [21].

In the clinical trials associated with Exa-Cel, adverse event management has been largely determined by the action's attendant on the busulfan conditioning regimen and any issues with the gene editing processes themselves. There is substantial evidence that the chemotherapeutic agent busulfan, now mainly utilized in the myeloablative conditioning regimen for Exa-Cel infusion, can cause potentially significant side effects. Grade 3 or 4 busulfan-associated side effects with serious respiratory and hepatic complications prevail among SCD patients taking part in clinical trials. What is important, however, is that most of such incidents seem to be rooted in the conditioning program as opposed to having any direct connection with Exa-Cel [21].

The most serious adverse events for SCD patients occurred in 20 patients, with some linked to busulfan medication. Defibrotide, a medication known to aid in such issues, was used to treat a patient who specifically had veno-occlusive liver disease. A terrible but distinct event that happened 268 days after the Exa-Cel infusion was the death of a patient with preexisting lung disease due to respiratory failure after contracting SARS-

CoV-2 [21]. The prevalence of serious adverse events highlights the necessity of cautious handling during the conditioning stage. Consequently, plerixafor is the sole medication used to mobilize stem cells in SCD patients in order to lower these dangers; while it is safer, the mobilization is less effective. Patients in both trials had extended neutropenia post-infusion and further reinforce that an engraftment process should be carefully monitored. The median time period in clinical trials for the neutrophil engraftment was 27 days for SCD patients and 29 days for transfusion dependent thalassemia (TDT) patients [21]. These delays underline the importance of keeping infections and other problems under hand during a sometimes-protracted recovery, even though these are to be expected with autologous transplantation. Remarkably, with protracted neutropenia, no deaths from bacterial or viral infections occurred during the studies, suggesting that supportive care measures to mitigate these risks were successfully implemented.

Overall, Exa-Cel has a positive safety record; most serious adverse events are linked to the conditioning program rather than the Exa-Cel infusion. At the data cutoff in April 2023, no cancer developments were noted, and no deaths directly linked to Exa-Cel infusion were reported in either TDT or SCD patients [21]. Ongoing treatment techniques, especially for immunological support and infection prevention, are crucial, nevertheless, as indicated by the extended neutropenia and the use of busulfan during the conditioning phase. Additionally, there were logistical issues with the harvest of stem cells in SCD patients, who needed more mobilization cycles than TDT patients. These issues need to be resolved further to improve patient outcomes. Although there is much

promise for Exa-Cel therapy for SCD, controlling side effects is a crucial part of care. To guarantee patient safety, the hazards associated with the conditioning regimen, stem cell mobilization, and engraftment procedures must be closely watched over and controlled. The results of the clinical studies show that these hazards may be successfully reduced with the right management techniques, opening the door for Exa-Cel to be widely used as a game-changing SCD treatment.

IV. COMPARATIVE ANALYSIS WITH EXISTING TREATMENTS

Hydroxyurea Therapy

Hydroxyurea has been the cornerstone of pharmacological treatment for SCD for many years. Since the FDA's approval for its clinical use in 1998, it mainly acts as a disease-modifying drug, it acts by enhancing fetal hemoglobin synthesis, inhibiting sickled hemoglobin from polymerization after deoxygenation. On the other hand, exagamglogene autotemcel-a CRISPR-Cas9-based gene-editing treatment-new strategy that targets the root cause of sickness.

The therapeutic effects of hydroxyurea are mediated by promoting the synthesis of HbF, which substitutes defective HbS in red blood cells. Through antagonizing the sickling process, HbF decreases hemolysis, vaso-occlusive events, and resultant organ damage. Myelosuppression is another side effect that gives a reason for the vigilant measurements - to maximize hydroxyurea's efficacy whilst minimizing its adverse effects, which

include myelosuppression and gastrointestinal upset [22]. On the other hand, Exa-Cel uses CRISPR-Cas9 technology to permanently alter the patient-specific hematopoietic stem cells. The gene-editing technique reestablishes HbF expression in RBCs through the activation of the BCL11A erythroid enhancer. This one-time dose reverses the genetic pathology and offers a prospect for functional cure. Unlike hydroxyurea, which alters the course of the disease without removing the underlying genetic flaw, this single-dose therapy addresses the underlying genetic pathology and offers the possibility of a functional cure.

Numerous studies have demonstrated that hydroxyurea lowers the incidence of VOsEs, hospital stays, and blood transfusion requirements [22]. However, not all patients benefit from it; others may not respond to it or have unbearable adverse effects. In order to prevent toxicity, prolonged usage necessitates rigorous adherence, frequent monitoring, and dose modifications. A systematic methodology directs the commencement and dose escalation of hydroxyurea to guarantee its safe and efficient usage. These crucial actions are described in [Table 2][22], which also highlights the significance of routine monitoring to optimize therapeutic benefits and minimize side effects.

Phase	Action/Recommendation
Pre-therapy laboratory tests	<ul style="list-style-type: none"> - Complete blood count (CBC) with differential (WBC, reticulocyte count, platelet count, RBC mean corpuscular volume). - Fetal hemoglobin measurement (if possible). - Renal and liver function tests. - Pregnancy test (for women).
Therapy initiation	<ul style="list-style-type: none"> - Baseline elevated fetal hemoglobin levels should not delay therapy initiation. - Counsel reproductive-age patients on contraception needs during therapy. - Start adults at 15 mg/kg/d (500 mg capsules); 5–10 mg/kg/d if chronic kidney disease is present. - Start infants/children at 20 mg/kg/d.
Monitoring and adjustments	<ul style="list-style-type: none"> - CBC with differential and reticulocyte count every 4 weeks when adjusting dosage. - Aim for absolute neutrophil count $\geq 2000/\mu\text{L}$; younger patients may tolerate counts down to 1250/μL. - Maintain platelet count $\geq 80,000/\mu\text{L}$.
Response to cytopenia	<ul style="list-style-type: none"> - If neutropenia/thrombocytopenia occurs: <ul style="list-style-type: none"> o Stop HU temporarily; o Monitor CBC weekly; o Reintroduce HU at 5 mg/kg/d lower dose once counts normalize.
Dose escalation	<ul style="list-style-type: none"> - If warranted, increase dose by 5 mg/kg/d every 8 weeks until mild myelosuppression (neutrophil count of 2000–4000/μL) is achieved, up to 35 mg/kg/d.
Long-term monitoring	<ul style="list-style-type: none"> - Regular safety checks every 2–3 months once stable dosing is achieved: CBC, reticulocyte and platelet counts. - Monitor RBC MCV and fetal hemoglobin levels for consistent response.
Additional considerations	<ul style="list-style-type: none"> - Clinical response may take 3–6 months; continue on max dose for 6 months before evaluating discontinuation. - Long-term therapy recommended even during hospitalizations or illness.

Table 2 Hydroxyurea treatment initiation protocol [22].

Exa-Cel has shown remarkable effectiveness in early trials, as evidenced by the notable decreases in VOEs and the achievement of transfusion independence by a considerable percentage of patients. One major benefit of the therapy is its longevity, as long-term HbF production yields ongoing clinical advantages. Its long-term safety profile and wider usefulness, however, are still being studied as a novel intervention [22]. Compared to more sophisticated treatments like Exa-Cel, hydroxyurea is widely available and reasonably priced. It is especially beneficial in environments with limited resources where access to state-of-the-art treatments may be limited. However, individuals and healthcare systems are severely burdened by the need for lifetime therapy.

Despite being revolutionary, Exa-Cel comes with a lot of financial and logistical drawbacks. Autologous stem cell transplantation, which is necessary for its

administration, calls for specialist facilities and interdisciplinary knowledge [22].

Although its curative potential could outweigh long-term healthcare expenditures for managing SCD, its high upfront price may limit its accessibility. Not all SCD patients can benefit from hydroxyurea, particularly those who are not responsive to myelosuppressive medications or who do not show clinical improvement. Furthermore, in areas with limited resources, its dependence on patient compliance and consistent medical follow-up poses difficulties.

For patients who are looking for a curative treatment or who do not benefit from hydroxyurea, Exa-Cel provides an alternative. But as of right now, it is only available to people who qualify for stem cell transplantation, not those who have serious comorbidities or little access to specialized care [22]. Careful patient selection and counseling are required due to the procedure's potential dangers, which include problems associated to myeloablation.

Despite representing different therapeutic paradigms, hydroxyurea and Exa-Cel are complementing rather than antagonistic treatments. For many SCD patients, hydroxyurea is still an essential choice since it provides a practical and efficient way to control the illness. A new age of curative medicines is ushered in by Exa-Cel, a groundbreaking gene-editing therapy that offers hope for a life free from the difficulties of SCD. When taken as a whole, these treatments highlight how crucial tailored therapy is to meet the various demands of the SCD community.

Stem Cell Transplantation

SCD, a genetic illness brought on by a mutation in the β -globin gene, has shown promise in response to stem cell transplantation and gene therapy. By replacing or altering the damaged cells that produce sickle hemoglobin, both treatments seek to offer a cure; yet they accomplish this through essentially distinct processes and methods.

Determining the best course of action for individuals with SCD requires an understanding of each therapy's mechanism of action, dangers involved, and possible results.

During a stem cell transplant (HSCT) donor stem cells are utilized to replace the patients damaged stem cells for treatment purposes. To minimize risks such, as graft versus host disease (GVHD) the donor cells can be sourced from either unrelated individual with a focus on achieving the compatible match. To prepare for the infusion of donor cells and facilitate their acceptance, a conditioning regimen is typically administered during the procedure to suppress the patient's system. The unhealthy sickle shaped hemoglobin is effectively substituted with the adult hemoglobin, by the donor cells once they integrate and begin producing blood cells; this helps alleviate the symptoms associated with SCD.

Although hematopoietic stem cell transplantation has shown effectiveness, in treating SCD there are risks associated with it. One major challenge is the availability of leukocyte antigen matched donors. There is still a chance of developing graft versus host disease and experiencing mortality related to the transplant procedure. Furthermore, the

preparatory conditioning regimens required prior, to the transplant may pose risks to organ health. Despite these risks involved with HSCT treatment, for SCD it has shown promising outcomes in the term. Studies on individuals undergoing HSCT, for SCD have reported a 91% survival rate and an 88% event free survival rate after 2 years [23].

For SCD, hematopoietic stem cell transplantation has generally been saved for patients who suffer from severe side effects, such stroke, or who are susceptible to long-term consequences [Table 3][24]. In clinical trials, stroke accounted for 57% of the first indications for HCT, while frequent vaso-occlusive pain crises accounted for 23%. Recent research, however, indicates that the most common reason for HCT is now recurrent episodes of pain exacerbations that necessitate medical attention. This change suggests that HCT is being viewed more and more as a way to enhance patients' quality of life (QoL) who are suffering from chronic pain, in addition to being a life-saving medication. The disease severity requirements for HCT consideration in SCD patients are shown in [Table 3][24], which also describes the progression of eligibility from a lifesaving intervention to a possible therapy that could improve quality of life. The decision-making process for identifying which patients would benefit the most from HCT is made clearer by the inclusion of these factors [25].

Stroke or CNS event lasting >24 h
ACS with recurrent hospitalizations or previous exchange transfusion
Recurrent VOC (≥ 2 /year for several years) or recurrent priapism
Impaired neuropsychological function and abnormal brain MRI
Stage I or II sickle lung disease
Sickle nephropathy (moderate or severe proteinuria or GFR 30–50% of predicted)
Bilateral proliferative retinopathy and major visual impairment in at least one eye
Osteonecrosis of multiple joints
Red cell alloimmunization (≥ 2 antibodies) during long-term transfusion therapy

Table 3 Disease severity criteria for consideration of HCT for SCD adapted from Walters et al [24]

Exa-Cel therapy, on the other hand, employs an autologous technique, which entails the harvesting, alteration, and reintroduction of the patient's own stem cells following genetic editing to express anti-sickling hemoglobin or elevated quantities of fetal hemoglobin. In order to compensate for the impaired adult hemoglobin, the gene editing procedure usually targets the BCL11A gene, which inhibits the generation of fetal hemoglobin and restores its expression. Through the use of CRISPR/Cas9 gene editing technology, Exa-Cel treatment alters the patient's HSCs outside of the body prior to transplantation.

This strategy avoids the hazards of GVHD by doing away with the necessity for a donor and may result in more individualized and easily available care. The risks of Exa-Cel may be lower than those of typical HSCT because it uses the patient's own cells, preventing immunological rejection, even if it also requires a myeloablative conditioning

regimen to assure effective engraftment. Exa-Cel's early clinical results have been encouraging, with patients reporting a notable decrease in SCD-related problems and improvements in symptoms such vaso-occlusive crises. However, there is currently little follow-up data available, and the long-term safety and effectiveness of Exa-Cel are still being assessed [23].

One of the primary distinctions between HSCT and Exa-Cel is the stem cell source. While Exa-Cel uses autologous stem cells, which removes the need for a donor, HSCT depends on allogeneic stem cells, which necessitate a well-matched donor. Finding an appropriate HLA-matched donor can be difficult, especially for patients from specific ethnic origins, therefore this distinction is extremely crucial. Furthermore, Exa-Cel therapy's autologous origin lowers the likelihood of GVHD, a frequent side effect of allogeneic HSCT. The type of genetic alteration is another significant distinction. While Exa-Cel directly modifies the patient's own cells to restore normal hemoglobin synthesis, HSCT just substitutes healthy donor cells for the damaged stem cells [23]. Both procedures necessitate conditioning regimens to prepare the patient's body for the transplant or infusion of transformed cells, but conditioning toxicity varies, with some concerns raised about the long-term effects of conditioning in both methods.

Despite these differences, both HSCT and Exa-Cel have shown promise in curing SCD, with considerable improvements in patient quality of life, including fewer vaso-occlusive crises and other SCD-related problems. The choice between HSCT and Exa-Cel is very

unique and is influenced by a number of variables, including the patient's age, the severity of their illness, the availability of a compatible donor, and their general health. Even though HSCT is still a proven treatment with years of clinical experience, Exa-Cel is a promising substitute, particularly for patients who don't have donor matches or don't want to take on the hazards of allogeneic transplantation. Both treatments may be used in the future to treat SCD, with developments in gene therapy possibly lowering risks and increasing accessibility. Ultimately, the comparison between HSCT and Exa-Cel underscores the evolving landscape of SCD treatment. As clinical trials continue to gather more data, both therapies may provide viable options for curing SCD, but each comes with distinct considerations that need to be carefully weighed by clinicians and patients alike [23].

Blood Transfusions

Among the various therapies for SCD, blood transfusions have been widely common as well. Blood transfusion has been used as a method to help lower the amount of HbS in the body. Having less HbS cells in the bloodstreams means RBCs are less likely to build up and block blood vessels. In a world where gene therapy is advancing, therapies like Exa-Cel have proven to be more effective in treatment. Each with their own mode of action and resulting data, Exa-Cel is a CRISPR/Cas9-based gene-editing therapy directed at editing the BCL11A gene in autologous hematopoietic stem and progenitor cells ex vivo in an effort to restore the production of fetal hemoglobin. This strategy produces anti-sickling hemoglobin in patients with transfusion-dependent thalassemia, a

type of SCD, and may be a long-term functional cure [26]. Symptom alleviation provided by supportive therapies, including blood transfusion, involves increasing the circulating number of healthy red blood cells and thus represents a very popular symptomatic treatment briefly relieving the symptoms of SCD. While blood transfusions relieve the results of sickling, they do not correct the underlying genetic cause of the disease and thus are often a continuous treatment.

A vital therapeutic option for SCD is red blood cell transfusion therapy, which helps manage the condition's many consequences. It improves oxygen-carrying capacity and overall vascular perfusion by lessening the load on sickled cells. The main indications for transfusion in SCD are compiled in [Table 4][27], which also highlights the particular problems for which this therapy is frequently used to lessen or avoid negative outcomes.

Indication	Type of transfusion
Acute transfusion indication	
Symptomatic anemia: aplastic crisis, acute splenic sequestration	Simple transfusion
Acute clinical stroke or TIA	Exchange transfusion
Acute hepatic sequestration/intrahepatic cholestasis	Simple or exchange transfusion
ACS	Simple or exchange transfusion
Acute multiorgan failure	Simple or exchange transfusion
Preoperative (surgeries lasting > 1 hour and require general anesthesia)	Simple or exchange transfusion
Pregnancy*	Simple or exchange transfusion
Chronic transfusion indication	
Primary stroke prevention	Simple or exchange transfusion
Secondary stroke prevention	Simple or exchange transfusion
Recurrent VOC	Simple or exchange transfusion

* Pregnant women with severe or frequent SCD-related complications or high-risk pregnancy.

Table 4 Indications for transfusion in SCD [27].

Regarding Exa-Cel, the CLIMB THAL-111 trial's findings indicate encouraging results for patients. With a median interval of 9 months since their previous transfusion, 42 out of 44 patients were able to cease receiving blood transfusions following Exa-Cel infusion. Furthermore, there was a notable rise in total hemoglobin levels, which persisted for more than a year [26]. Given that 16 patients avoided transfusions for at least a year, the treatment showed promise as a long-term fix. This is in contrast to blood transfusions, which, although they are useful for short-term symptom management, frequently necessitate lifelong care and can result in problems like iron overload and the requirement for iron chelation therapy.

There are dangers associated with both approaches. Although these were addressed without long-term problems, Exa-Cel therapy has been linked to significant side effects such hemophagocytic lymphohistiocytosis and delayed engraftment. It also requires a myeloablative conditioning regimen that includes busulfan. Conversely, infusions of blood carry the danger of iron overload, transfusion responses, and alloimmunization [26]. The primary distinction is that, whereas transfusions continue to be a lifetime, symptomatic treatment, Exa-Cel has the potential to address the underlying cause of SCD in the long run by offering a one-time, potentially curable therapy.

Although short-term blood transfusions are useful for controlling SCD symptoms, Exa-Cel therapy presents a novel, potentially curative strategy that may be able to do away with the requirement for continuous transfusions and provide long-term increases in hemoglobin levels. An important development in SCD treatment is Exa-Cel's capacity to offer long-term advantages with a single infusion [26].

V. QUALITY OF LIFE AND LIMITATIONS

Patient-reported Outcomes

Based on the findings of the CLIMB TDT-111 study report mentioned by researchers and experts, in the field of medicine and genetics research area. A cutting-edge gene editing therapy called Exa-Cel that utilizes CRISPR technology presents potential in improving the quality of life for individuals suffering from transfusion

dependent β thalassemia condition. Various standardized assessments like 55LD-55F were applied during this research project involving both grownups and teenagers aged between 12 to 35 years old to gauge enhancements, in patient reported health outcomes (PROs) [28]. The results indicated that the quality of life, for both age groups had significantly improved from a standpoint.

By the end of the year of the study period the average increase, in the EQ health score for adults had gone up from an initial 0.85 to 0.12 exceeding the minimum significance level of 0.078 [28]. The FACT-G wellbeing assessment indicated improvements in aspects such as function, emotional wellbeing, social interactions, and physical health with an average increase of 10.3 points by the end of month 24 well, above the significant threshold. Moreover, an enhancement of 6.8 points, in the rating system for Bone Marrow Transplants (BMTs) suggests improved health and wellbeing in connection, to the transplant process and related physical health aspects.

Older adolescents achieved a mean improvement of 4.8 points in the EQ VAS between the baseline and month 18. Furthermore, the mean scores of the Pediatric Quality of Life Inventory (PedsQL) yielded an improvement of 14.1 points of life quality measured in total youth population, which included functioning (19.2 points) and psychosocial health (11.4 points) [28]. The findings of TDT study not only affirm Exa-Cel's ability to address the clinical need for TDT treatment, but also indicate broader physical and psychosocial well-being benefits that are improved. Results from the CLIMB TDT-111 study highlight the wide-ranging and sustained HRQoL benefits with Exa-Cel treatment in adult and

adolescent patients, further standing to reinforce the potential of Exa-Cel to transform the lives of individuals living with TDT through alleviating the burden caused by recurrent blood transfusions and their complications [28]. These results further disseminate awareness of Exa-Cel as a safe, efficacious therapeutic alternative that enhances both clinical as well as patient-reported outcomes in sickle cell patients with TDT.

Results from the phase III CLIMB SCD-121 trial exhibited a reduction in frequency of vaso-occlusive crises and hospitalization for Exa-Cel, with 97% of participants remaining VOC free for over 12 months following treatment. Results indicate that all patients showed an early response to the therapy, with Hb levels remaining improved long-term, with near normal Hb achieved by six months post-infusion. These findings, particularly given the reduced risk of graft-versus-host disease [29], indicate that Exa-Cel has similar efficacy to classic treatments including allogeneic hematopoietic cell transplantation. Compared to older methods, such as blood transfusion, Exa-Cel offer a number of advantages. Because Exa-Cel uses autologous cells, the risk of immune-related complications is extremely low due to the absence of immune-matching. Although this therapy necessitates myeloablative conditioning, the regimen is much less intense than that of other methods as it only incorporates chemotherapy [29]. In fact, Exa-Cel has the potential to be a functional cure by targeting and eliminating the underlying issue of SCD through increasing levels of HbF with a single treatment. That is a huge advance from continuous, life-long blood transfusions which do not resolve the underlying gene mutations that causes the disease.

The concern around myeloablative conditioning, with often long stays in hospital and significant risk of infections, is offset by the potential advantages of Exa-Cel therapy to drastically decrease VOCs over time and may offer an improved standard of life compared to other conventional therapies including transfusions or alloHCT. While Exa-Cel offers these benefits, availability of this therapy is limited because of the time to collect and process cells and possible insurance challenges [29]. Nevertheless, the longer-term durability and safety of Exa-Cel is also under assessment in long-term studies following patients by means of an annual physical for up to 15 years. So far so good: there have been no malignancies in the Exa-Cel cohort, differing from what has happened with other gene therapies such as lovetibeglogene autotemcel which carries a black-box warning on malignancy. These preliminary results are exciting and indicate the potential of Exa-Cel to transform the treatment landscape for patients with SCD and other hemoglobinopathies, however widespread implementation may not occur just yet due to logistical and economic barriers.

Accessibility and Affordability

Exa-Cel has demonstrated the potential as a transformative gene therapy to address SCD by reducing severe vaso-occlusive crises, improving quality of life, and providing understand benefits to patients. Nevertheless, as this therapy makes the move to clinical practice, its availability and price are important factors. SCD is a debilitating disease which burdens significantly not only the patients but also the healthcare system. The cost of SCD care is substantial, averaging an estimated \$3 billion per year in direct

medical expenses just in the U.S. These costs include that of repeated blood transfusions, hydroxyurea treatment and recurrent hospitalization for pain management—all can be reduced with successful Exa-Cel which provides the promise of a potential cure through a single one-off intervention [30].

Although Exa-Cel provides a durable reduction in disease-related morbidity and has proven quality of life (QoL) benefits, the estimated \$2 million price tag per patient will limit its availability to wealthy patients or community centers. Exa-Cel costs so much as their multistep process of obtaining the cells, genetically modifying them, and then infusing a new version of those cells involves specialized technology and infrastructure. Providing long-lasting effects and eliminating the need for continued care such as blood transfusions and pain relief, the upfront cost of the gene therapy may be prohibitive for many patients—especially those with poor insurance coverage or living in areas lacking specialized treatment [30].

The cost-effectiveness of the treatment has also been challenged; according to ICER, at a price point of \$2 million per therapy, this translates into incremental costs of \$170,000-220,000 per quality-adjusted life year (QALY) gained—which is viewed as a high cost in healthcare contexts. For many healthcare systems — especially in poorer nations, or those communities with little access to advanced medical technologies — these expenses may not be long-term viable. Nevertheless, Exa-Cel continues to be one of the most effective choices for patients who meet the criteria for treatment and have a scarcity of

alternative potentially curative treatments as allogeneic hematopoietic stem cell transplantation.

A cost-effectiveness analysis of Exa-Cel compared with standard care, based on incremental costs and health outcomes, was conducted using a model developed by the Institute for Clinical and Economic Review. The outputs of this analysis, [Table 5][30] demonstrate the value for money associated with Exa-Cel versus usual care for SCD.

Treatment	Treatment cost ^a	Total cost	VOCs	Life years	evLYs	QALYs
Health care system perspective						
Lovo-cel or exa-cel	\$2,000,000	\$2,827,000	4.18	21.87	17.31	16.38
Standard of care	—	\$1,490,000	119.26	15.80	9.44	9.44
Incremental cost-effectiveness ratios	—	—	\$11,600	\$220,000	\$170,000	\$193,000
Modified societal perspective						
Lovo-cel or exa-cel	\$2,000,000	\$2,837,000	4.18	21.87	17.31	16.38
Standard of care	—	\$1,714,000	119.26	15.80	9.44	9.44
Incremental cost-effectiveness ratios	—	—	\$9,800	\$185,000	\$143,000	\$162,000

^a Placeholder price of \$2 million.

evLY = equal-value life-year; exa-cel = exagamglogene autotemcel; lovo-cel = lovotibeglogene autotemcel; QALY = quality-adjusted life-year; VOC = vaso-occlusive crisis.

Table 5 Results and Incremental Cost-Effectiveness Ratios for the Base Case of Lovo-cel and Exa-Cel vs Standard Care [30].

Access to Exa-Cel will require health care policies to change, including consideration of insurance and pricing systems that work for both the payer and patient in order to enable access within broader segment of patients. Part of making sure the benefits of Exa-Cel

are actually experienced includes tackling the inequities built into healthcare that prevent racial and societal access to new, advanced treatments for SCD. The innovation is indeed promising, but its availability and affordability are contentious issues, especially for low- and middle-income countries (LMICs). Here, we analyze the implications of Exa-Cel for global health equity, its potential costs, and the underlying systemic barriers to uptake.

The cost of Exa-Cel, estimated to be around \$2 million per infusion, can also create a major hurdle towards affordability. For the majority of people, especially in LMICs, this financial expenditure is unattainable. Underpinning these costs are the complexity of CRISPR-Cas9 delivery modalities, substantial R&D investment, and manufacturing processes necessary for autologous therapies. Although they are effective in RNAs transfection, RNP delivery methods require expensive reagents and technologies. Alternatively, plasmid cloning is less expensive but requires an elaborate and time-consuming procedures with expert capability [31]. These costs add up quickly and make the rationale for using Exa-Cel in all but a few well-funded healthcare systems difficult to understand. In LMICs, where science and technology budgets are typically already less than 1% of GDP, these costs are impossible to absorb without massive subsidy or overseas assistance. In high-income countries, inequities in insurance coverage and healthcare financing can limit access for low-income populations. Unless a cost-reduction strategy or funding mechanism is identified to lower the prices of Exa-Cel, it risks becoming an ultra-orphan drug with limited benefit, available only to and for the richest nations or individuals. Exa-Cel requires specialized facilities, trained staff and ongoing

care after treatment is complete. In lower-middle-income countries, these resources are limited and the potential unrealized due to weak healthcare systems and insufficient local training in genome editing technologies.

The expertise to develop and roll out CRISPR-Cas9-based therapies, such as nucleofection methods followed by quality control—desirable knowledge rarely present in locations outside the Global North [31]—requires highly specialized professionals. During your analysis of the accessibility barriers, you must note that the choice of CRISPR-Cas9 delivery formats and enrichment strategies is not without significance. Methods differ in cost, efficiency, and technologic complexity with its clear implication for resource poor settings. Analyzing data from the results section and tabulated in [Figure 8][31] provides a competitive overview of these common delivery formats and

demonstrates their approximate cost-effectivity when compared to both the original and overexpressed genes used as shuttles towards engineering improvements.

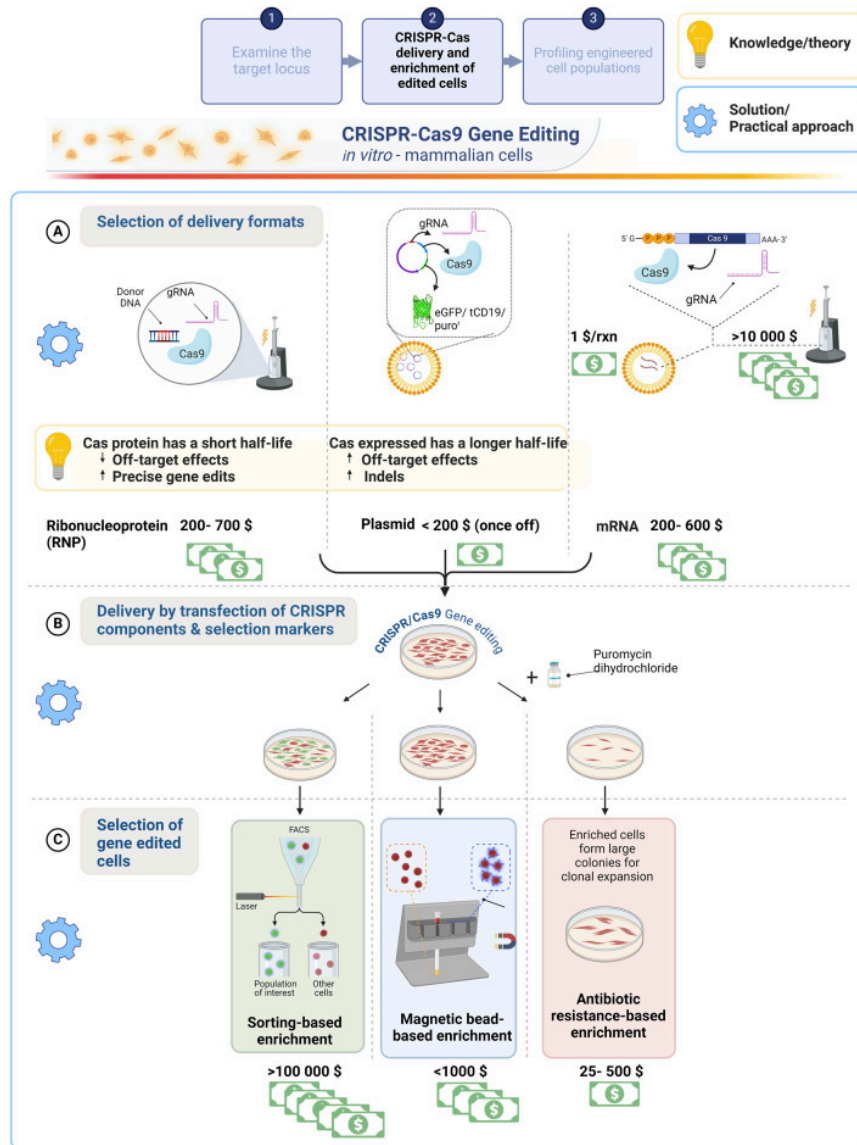


Figure 8: A comparative overview of common CRISPR-Cas9 delivery formats [31].

Previous research has demonstrated that CRISPR landmark discoveries cluster in HICs with the potential to deprive researchers in LMICs of exposure to cutting edge science.

That imbalance only lengthens the lag of technology transfer and continues inequity building another cycle. While local infrastructure and resources might stymie efforts to make Exa-Cel widely available, even with international collaborations. Tackling these challenges necessitates a comprehensive approach. Without collaboration between high- and low-income countries, Exa-Cel will not be accessible to many across the globe. This can be accomplished by increasing funding to LMICs, implementing technology transfer programs, and creating regional centers of excellence in genome engineering. As an example, reagents, such as plasmid purification kits, are much cheaper to produce locally and Frugal labs around the world have shown that high-level capabilities can be realized by using in-house methods [31]. If Exa-Cel is to deliver on its promise as a curative therapy for SCD, stakeholders need to eliminate the systemic barriers that restrict access. Efforts to promote worldwide cooperation, develop domestic capacity and advocate for equitable and just policies are crucial so that genome editing technologies can serve all of humanity, regardless of geographic or economic circumstance.

CONCLUSION

The FDA-approved Exa-Cel is a game-changer in therapy for SCD because it ultimately targets the genetic source of this debilitating disease. Yet, this milestone represents only the initial step along a wider path towards perfecting, broadening, and making accessible that next-generation treatment. An important next step will be to make Exa-Cel more scalable and accessible in order to treat patients in the context of resource-constrained health systems. Although the current *ex vivo* process works well, it relies on dedicated infrastructure and very high costs, which limits treatment accessibility for most patients in low- and middle-income countries where SCD burden is highest. If successful, recently discovered *in vivo* gene-editing techniques may enable more efficient delivery without complex protocols and enhance the feasibility and affordability of this therapy worldwide. Safety and durability of effect remain among the top priorities. Although recent trials indicate Exa-Cel is well tolerated, patients require ongoing observation of any long-term complications. As the precision of gene-edits improves even further, these risks will also be reduced, and therapy made more confident. Simultaneously, the measurement of patient-reported outcomes like health-related quality of life and day-to-day functioning will be necessary to fine-tune the treatment while also meeting broader aspects of patient needs.

The importance of continued investment in gene-editing research is also underscored by the success of Exa-Cel. As exemplified by BCL11A targeting to reactivate fetal hemoglobin, foundational discoveries highlight the power of scientific curiosity in

leading to transformative clinical applications. We will build on this with further innovations, such as expanding the reach of gene-editing therapies from SCD into additional genetic diseases. Exa-Cel is more than a treatment—it is progress, hope and the promise of genetic medicine. Although work continues enhancing accessibility, improving safety, and scaling it up for more diseases, this breakthrough brings us a step closer to a time where diseases such as SCD can be effectively treated in an equitable manner. With continued innovation and investment in this space, we are getting closer to a world where transforming therapies such as Exa-Cel will become available for everyone who needs it.

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Vita

