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Crispr-Cas9: Revolutionizing Targeted Drug Delivery for Sickle Cell Anemia Therapy

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ABSTRACT
Sickle Cell Anemia SCA is a severe genetic disorder involving point mutations in the HBB gene encoding haemoglobin beta, which leads to abnormal haemoglobin and subsequent red blood cell sickling. Recently developed CRISPR-Cas9 gene editing has opened up new avenues for its targeted therapies. In the case of SCA, this CRISPR-Cas9 edits the HBB at precise locations. On the fronts of clinical success, two such next-generation treatments that are at the top of the race include Casegvy and Lyfgenia by CRISPR Therapeutics. These gene therapies are known to reprogram hematopoietic stem cells so that the production of healthy haemoglobin can be produced, hence potentially able to cure SCA and demonstrate the transformative power of CRISPR in precision medicine.

Keywords: *Sickle Cell Anemia, CRISPR-Cas9, Haemoglobin, Casegvy, Lyfgenia*

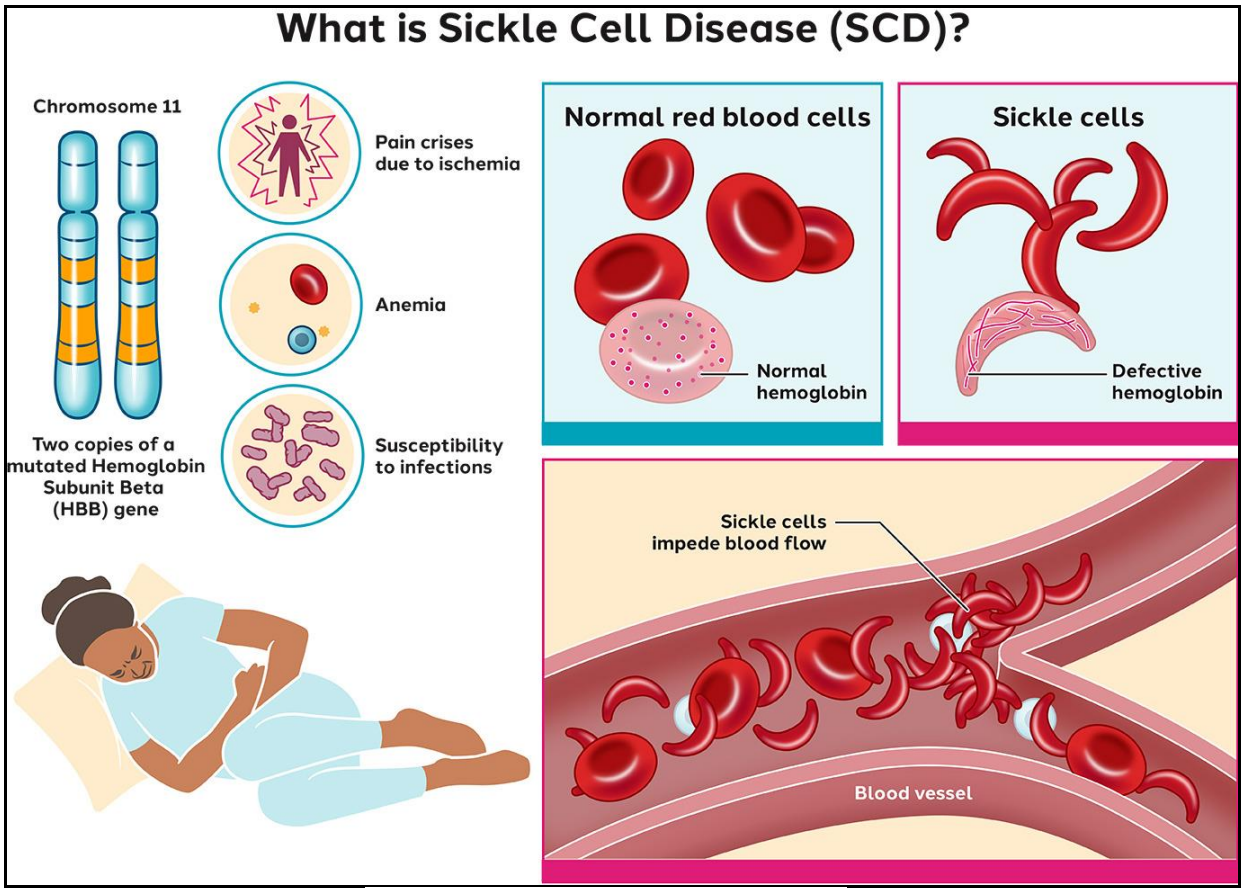
INTRODUCTION
Sickle Cell Anemia Overview: Sickle cell anemia is a hereditary blood disorder caused by a mutation in the β-hemoglobin gene, leading to the production of sickle hemoglobin (HbS). This condition is characterized by the polymerization of deoxygenated HbS, which results in the sickling of red blood cells (RBCs), vasoocclusion, and hemolytic anemia. The disease exhibits a diverse phenotype influenced by various genotypes, including homozygosity for HbS and compound heterozygosity with other hemoglobin mutations. Key factors affecting the severity of sickle cell anemia include fetal hemoglobin (HbF) levels, which can inhibit HbS polymerization and mitigate symptoms. [1]

There is an urgent need for innovative therapies to improve patient outcomes in sickle cell disease, as there is currently a considerable lack of knowledge regarding determinants of SCD severity and few reliable objective laboratory tools for risk stratification in daily management. Additionally, the existing treatments, such as hydroxyurea, have limitations, including significant side effects and the fact that approximately 40% of patients do not respond to it at all. Therefore, the identification of new risk factors and objective markers for monitoring patients, as well as the development of safer and more widely applicable therapeutics, is essential. [2]

CRISPR's Promise
CRISPR technology offers precise gene editing capabilities through the use of a specific RNA (guide RNA) sequence that recognizes the target DNA region of interest and directs the effector Cas protein for editing. This system is relatively easier, cheaper, and more efficient compared to conventional genome engineering methods, which are costly and require significant expertise. The high specificity of the nucleases used in CRISPR reduces off-target

effects, enhancing the precision of gene editing. Additionally, advancements in CRISPR technology, such as double nicking and the use of truncated guide RNAs, further improve the specificity of genome editing. [3]

CRISPR technology has significant potential impact on treating genetic diseases, particularly monogenic disorders like sickle cell disease (SCD). It offers a promising approach for correcting the underlying mutations responsible for such diseases, which could lead to permanent removal or correction of detrimental mutations. This capability positions CRISPR as a valuable tool in gene therapy, potentially providing a safer and more effective cure for patients who currently lack suitable treatment options. The ongoing clinical trials and proof-of-principle studies demonstrate the feasibility of using CRISPR/Cas9 to induce therapeutic effects, such as the correction of SCD mutations or the induction of fetal hemoglobin expression, thereby moving closer to realizing effective treatments for genetic diseases. [3]



Understanding Sickle Cell Anemia[28]

Understanding Sickle Cell Anemia

Genetic Basis: The specific mutation causing abnormal red blood cells in sickle cell anemia is a point mutation in the β-hemoglobin gene (HBB). This mutation involves a substitution at codon 6, where the normal codon for glutamic acid (GAG) is replaced by the codon for valine (GTG). This change results in the production of sickle hemoglobin (HbS), which has the unique property of polymerizing when deoxygenated. [1]

The polymerization of deoxyHbS leads to the deformation of red blood cells into a sickle shape, which is a key characteristic of the disease. These sickle-shaped cells are less flexible and can obstruct blood flow in small vessels, leading to vasoocclusion and various

complications associated with sickle cell disease. Additionally, the sickling process injures the erythrocyte membrane, resulting in a heterogeneous population of red blood cells, many of which are short-lived and prone to haemolysis. [1]

Overall, this single point mutation has profound effects on the structure and function of hemoglobin, leading to the clinical manifestations of sickle cell anemia. [1]

Sickled cells restrict blood flow primarily through their altered shape and increased adhesion properties. The sickle-shaped red blood cells are less flexible than normal red blood cells, making it difficult for them to navigate through small blood vessels. This rigidity can lead to vasoocclusion, where the sickled cells obstruct blood flow, particularly in the microcirculation. Additionally, sickle cells have a tendency to adhere to the endothelium of blood vessels, which further exacerbates the blockage of blood flow. Stress reticulocytes, which are produced in response to the anemia caused by sickle cell disease, are the first to adhere in the microcirculation, facilitating the entrapment of other erythrocytes and leukocytes, leading to increased blockage and inflammation. [1]

The resulting vasoocclusion can cause acute pain crises, as tissues become ischemic due to lack of oxygen. Over time, repeated episodes of vasoocclusion can lead to organ damage due to chronic hypoxia and inflammation, affecting organs such as the spleen, kidneys, and lungs. [1]

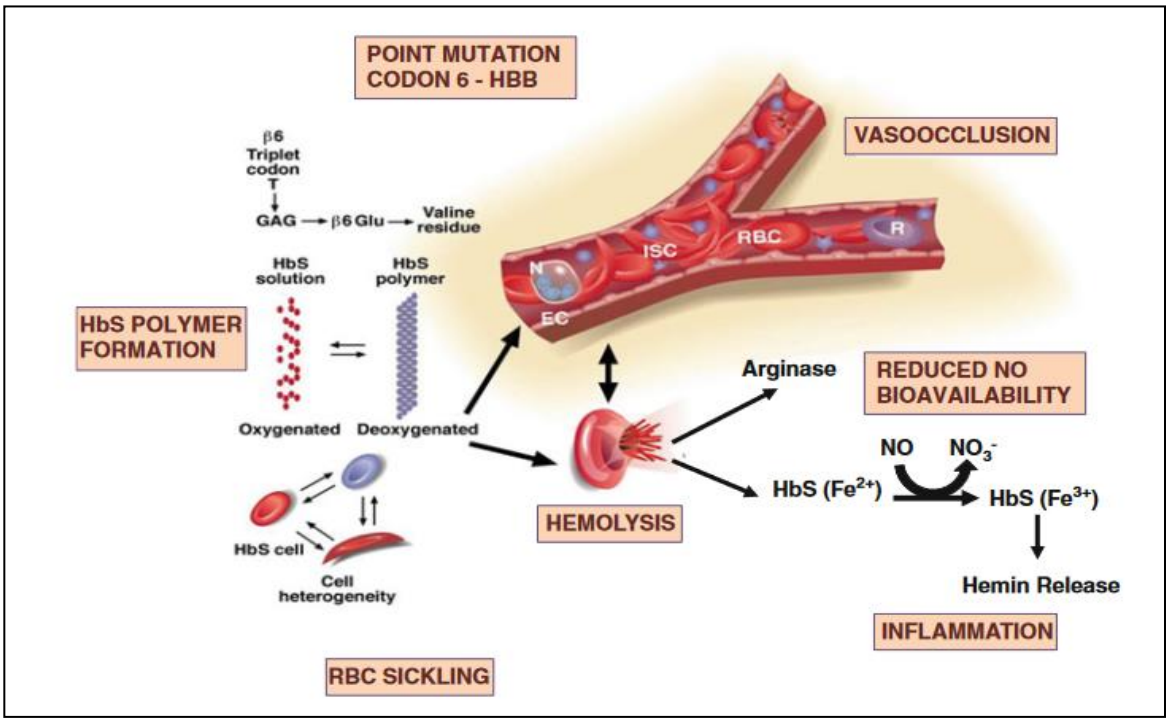
Clinical Challenges

Vaso-occlusive events (VOEs) in sickle cell disease (SCD) occur due to the entrapment of sickled cells in the microcirculation, leading to tissue ischaemia and damage in almost all organs. This process accounts for the majority of SCD-related complications, and the affected vascular bed determines the clinical presentation of these events. [2]

The most frequent cause of hospitalization in sickle cell patients is the painful crisis, which is a result of microvascular occlusion of the bone marrow. This occlusion leads to tissue ischaemia, causing excruciating pain that often necessitates hospital admission and treatment with opioid analgesics. Pain is commonly located in the lumbar spine, femora, ribs, and sternum, as well as in the abdomen, where the aetiology of 'abdominal crises' is not clearly understood but can be mistaken for acute abdominal problems requiring surgical intervention. [2]

Additionally, vaso-occlusion can lead to various complications, including painful skin ulceration, which is particularly common in patients with homozygous SCD. This complication has a peak incidence during the second and third decades of life, affecting up to 75% of adult HbSS patients. The poor healing tendency associated with these ulcers is significantly influenced by vaso-occlusion, although it is not the sole aetiological factor. [2]

Overall, VOEs significantly impact patient quality of life, leading to recurrent pain crises, increased hospitalizations, and long-term complications that can affect multiple organ systems. [2]



Vaso-occlusive events (VOEs) in sickle cell disease (SCD)[29]

Current treatments for sickle cell disease

The treatments particularly hydroxyurea, have several limitations:

1) **Response Variability:** Approximately 40% of patients do not respond to hydroxyurea treatment at all, which limits its effectiveness as a universal therapy for all sickle cell patients.
2) **Side Effects:** Hydroxyurea is associated with significant side effects, including myelosuppression and leg ulceration. There are also concerns regarding the potential risk of malignancies with long-term exposure to hydroxyurea.
3) **Limited Treatment Options:** The proven effective treatment options for sickle cell patients are limited primarily to hydroxyurea, blood transfusions, and bone marrow transplantation. This lack of diverse therapeutic options can hinder optimal patient management.
4) **Complications Despite Treatment:** Major complications can still occur in both children and adults despite hydroxyurea therapy, indicating that it does not completely prevent the disease's complications.
5) **Need for Risk Stratification Tools:** There is a considerable lack of reliable objective laboratory tools for risk stratification in the daily management of sickle cell disease, which complicates the treatment process and may lead to suboptimal care.

These limitations highlight the urgent need for innovative therapies to improve patient outcomes in sickle cell disease.

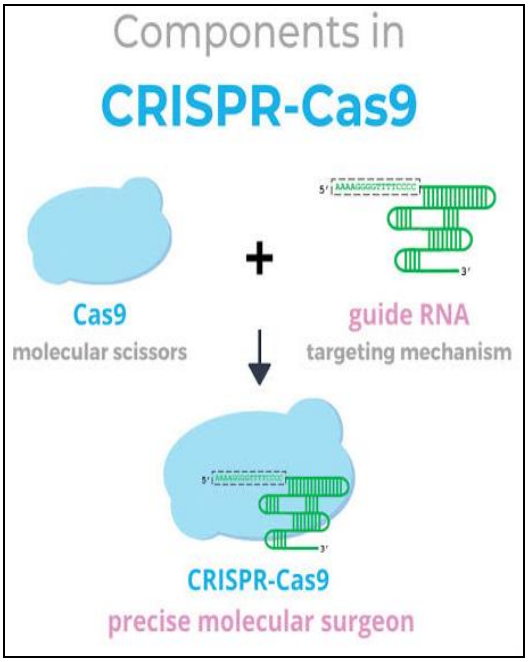
CRISPR-Cas9 Basics

Genome Editing with CRISPR

1. CRISPR-Cas9 System: The CRISPR-Cas9 system is, in fact, a form of adaptive immune system derived from bacteria and archaea. It combats virus infections by cleaving foreign

DNA. More recently, it had been repurposed within the domain of biotechnology for genome editing of a wide range of organisms, from plants and animals to humans. The main players in this system are the protein Cas9 and a second RNA molecule, termed a guide, which is correspondingly designed as single-guide RNA (sgRNA).[4][5]

2. CRISPR-Cas9 System Components



CRISPR-Cas9 System Components[30]

- **Cas9 Endonuclease:**

Cas9 is a DNA endonuclease directed by RNA. According to the sgRNA's determination, it makes particular cuts to the DNA. There are two distinct types of nuclease-active domains found in the Cas9 enzyme. HNH and RuvC are these. The RuvC domain cleaves the DNA strand in opposition to the sgRNA, whereas the HNH domain cleaves the complementary DNA strand, creating a double-stranded break (DSB)[6]

- **sgRNA, or single-guide RNA:**

A unique RNA sequence called the sgRNA directs Cas9 to the desired DNA location. This RNA molecule is a hybrid of two naturally-occurring RNA molecules: trans-activating crRNA and CRISPR RNA. The sgRNA's complementary crRNA portion maintains that the Cas9 complex with the target DNA sequence Cas9 with the sgRNA will bind precisely at the target location in the genome.[7]

- **Protospacer Adjacent Motif (PAM)**

For the Cas9 protein to work, the short DNA sequence close to the target site needs to be present. The commonly used Streptococcus pyogenes Cas9 has the PAM sequence "NGG," where "N" can represent any nucleotide. The binding of Cas9 to the target DNA and the ensuing cleavage depend on this region.[8]

3. Mechanism of DNA targeting and cleavage

- Target recognition: The sgRNA guides the Cas9 protein to its exact DNA target by base pairing with the matching DNA sequence. As a result, Cas9 experiences a conformational shift that results in an active site for every nuclease domain. The HNH domain cuts the

sgRNA's complementary DNA strand, but not the opposing strand, resulting in a double-stranded break (DSB).[4]

- Cas9 Nuclease Domain Activation and DNA Cleavage: In the presence of the target DNA bound to the Cas9 protein and the recognition of a PAM sequence, the nuclease domains are activated. This process produces a DSB at the target with high precision. A given break can then be further used for the introduction of genetic modifications by the natural pathways of the cell.[5]

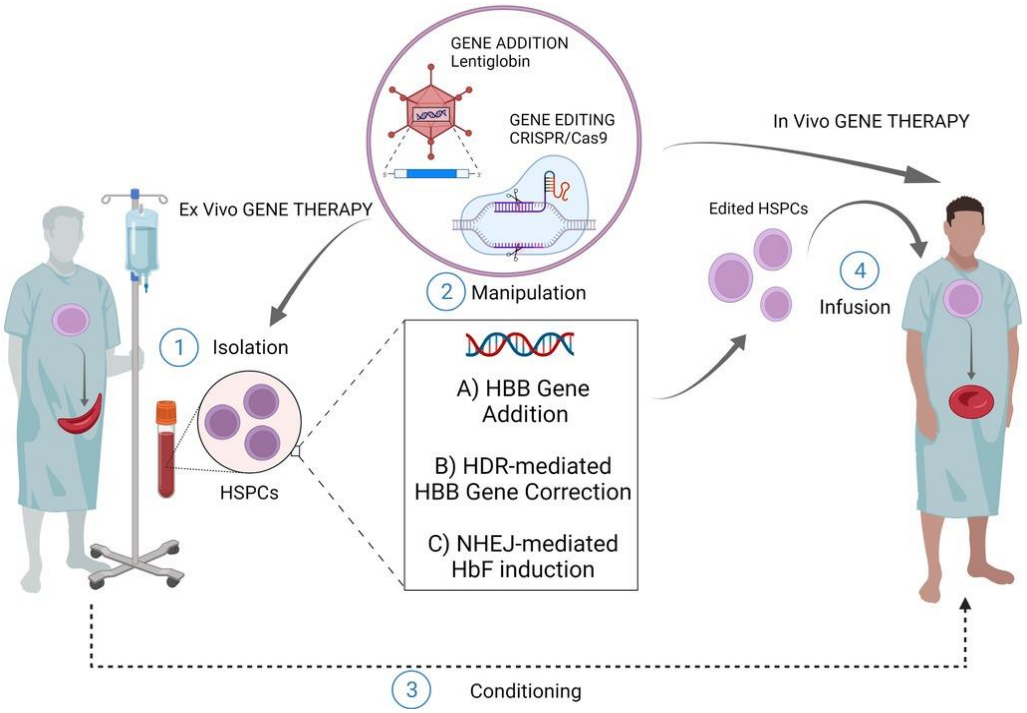
4. Cleavage-activated DNA repair pathways: After the Cas9-induced DSB, the cell uses one of two major DNA repair pathways to repair the break. The choice of which one is used dictates the genome editing outcome.

- **Non-Homologous End Joining:** Among them, NHEJ is the major and most efficient mechanism of DNA repair in cells. In general, it directly joins the broken DNA ends to repair DSBs without a homologous template. Since the process is error-prone and frequently leaves an indel, an insertion or deletion of a few nucleotides at the site of repair, which may disrupt the reading frame of a gene and result in loss of function or "knockout" of the target gene, this could be true.[6]
- **HDR: Homology-Directed Repair.** HDR is a high-fidelity, low-efficiency repair pathway that uses a homologous sequence of DNA as a template for repair and thereby repairs accurately. It can be exploited to achieve specific genetic edits by provision of a synthetic DNA template carrying the intended sequences. Normally, it is only active during the S and G2 phases of the cell cycle, once the genome has been duplicated, so a sister chromatid may serve as a template for repair. [7][8]
- **Ensuring Accuracy:** The accuracy of the CRISPR-Cas9 technique has much to do with how specific the sgRNA is against its target sequence. Off-target effects are a possible threat where the Cas9 nuclease cuts non-intended sites; however, advancements in the design of sgRNAs and construction of high-fidelity variants of Cas9 have dramatically reduced such risks. This research was developed to further improve genome editing accuracy by eliminating off-target activity using techniques with paired nickases or improved Cas9 proteins. [9]

5. Advanced Applications

- **Multiplexed Genome Editing:** As the CRISPR-Cas9 system is capable of supporting multiple sgRNAs against **Multiplexed Genome Editing:** different genes in the same experimental setting, such features are really quite useful in studying gene-gene interactions that lead to genetic diseases. This ability is very useful for generating models of complicated diseases and performing high-throughput genetic screens. For example, many sgRNAs designed against various exons in a gene can cause large deletions or other types of genetic alteration. [9]
- **Priming and Base Editing:** Base editing and prime editing represent two applications that have been recently developed with this fast-developing CRISPR technology. Base editing allows the direct modification of a DNA base without the formation of double strand breaks. While base editing may potentially decrease the introduction of unwanted mutations, its application in editing is presently severely curtailed. Prime editing employs an HDR-independent method to perform more complex genetic editing at a specified region of DNA—for example, insertion or deletion—by the precision of CRISPR with the action of a reverse transcriptase enzyme.[9][10]

It has propelled genetics into a revolution by the robust, adaptive, comparatively easy-to-use genome editing tool that is known as CRISPR-Cas9. This system causes targeted breaks in DNA and then recruits cell intrinsic mechanisms of repair to effect a given genetic change, such as the introduction of a certain sequence or knockout. Still much learning is left to do, but applications of this technology are growing fast and opening up new avenues for agriculture and medicine.



CRISPR in SCD[31]

Besides, the fact that the system of CRISPR-Cas9 can induce precise breaks in the DNA and utilize cellular mechanisms of repair to effect certain genetic changes has revolutionized genetic engineering. Research, medicine, and biotechnological applications have increased as technology changes, offering new ways in which the knowledge toward genetic diseases and treatment expands

Modified Blood Stem Cells: Besides, the fact that the system of CRISPR-Cas9 can induce precise breaks in the DNA and utilize cellular mechanisms of repair to effect certain genetic changes has revolutionized genetic engineering. Research, medicine, and biotechnological applications have increased as technology changes, offering new ways in which the knowledge toward genetic diseases and treatment expands.

1. Collection and Isolation of HSCs

Hematopoietic stem cell (HSC) are responsible for the production of all blood cells. They're generally deduced from the bone gist; still, they could also be mustered into supplemental blood using granulocyte- colony stimulating factor. rallying increases their yield within the circulating blood for easier collection using apheresis. It's a minimally invasive fashion that provides a robust source of cells to be used for gene editing. The collected cells suffer sanctification in order to enrich the population of the true HSCs that are specifically linked only by particular face labels similar as CD34.[11]

2. Editing Gene via CRISPR-Cas9

Targeting individual genes:

The CRISPR-Cas9 editing system is used to make changes at specific genes in the harvested HSC. For instance, in SCD treatment, it targets the BCL11A gene known as a regulator of HbF expression. On disruption, BCL11A has been found to increase the level of HbF significantly enough to replace defective adult haemoglobin and be efficacious in attenuating SCD symptoms. Such approaches exploit the inherent compensatory mechanisms in haemoglobin regulation to exert their therapeutic effects. [11][12]

Mechanisms of CRISPR: It includes the use of the CRISPR-Cas9 system consisting of the Cas9 endonuclease and a single guide RNA, which directs Cas9 to a specific target gene by base complementarity with the DNA sequence. The binding of Cas9 induces a double-strand break at the target site within the gene. This double-strand break is repaired through the Non-Homologous End Joining or the Homology-Directed Repair pathway of the cell's endogenous DNA repair process. In contrast, NHEJ is an error-prone process that generally causes small insertions or deletions that create an indel, thus mostly resulting in gene disruption. HDR is a precise gene editing or insertion technique in the presence of a repair template and is therefore suitable for the introduction of specific genetic modifications. [11][14]

Editing Efficiency Enhancement: Gene-specific gRNA designs by high-fidelity Cas9 variants, delivery strategies that briefly express the CRISPR-Cas9 hardware in HSCs, to maximize editing efficiency and reduce off-target alterations to HSC genomes. These will be critical in allowing the optimization of the hARNCT platform to enable the creation of therapeutic product candidates that are safe and effective. These optimization processes will thus be essential to ensure that the modified cells are safe and effective as well.[15]

3. Expanding and Checking Rewritten HSCs

Mutation Testing: The extent of the desired genomic alterations introduced by the CRISPR editing in the genetically transformed HSC was scrutinized. Some of the methods for doing this include digital droplet PCR and next-generation sequencing, which are used for qualitative assessment of intended edits and detection of off-target effects that impair the safety and efficacy of the therapy, respectively. [14]

Expansion: The successfully modified HSCs are then expanded in vitro until sufficient cells to be used for transplantation are obtained. Indeed, at this stage, the stem cell's properties are still maintained while the cells proliferate on a specialized medium. As such, this form of growth becomes imperative in order to produce adequate numbers of genetically modified cells that will confer therapeutic benefits following transplantation. [11][12]

Functional Validation: in vitro tests and in vivo models are used to test the functionality of the modified HSC population. These assays quantitate their engraftment potential and ability to generate different blood lineages and reconstitute the bone marrow—precisely the essential properties that should not be affected by the gene edits. In reality, functional validation is required to ensure that the patients' modified cells engraft and ultimately generate healthy blood cells.[15]

4. Patient Conditioning

Before the modified HSCs are introduced into the patient, he or she undergoes a conditioning program to help prepare his or her bone marrow to accept the new cells. This can be done, typically, through radiation therapy, chemotherapy, or a combination of both. Such a regimen has a twofold purpose: it dampens the immune system of the patient so that his or her body

does not reject the newly introduced cells, and it creates space in the bone marrow by removing resident cells. This is particularly true for the conditioning programs of patients with pre-existing diseases; the intensity is calibrated to be effective against the possibility of side effects. [14][16]

5. Edited HSCs Transplantation

These edited and expanded HSCs are injected through intravenous injection directly into the patient's blood stream. After administration, the cells automatically home back to the bone marrow, engraft, and start the process of new blood cell formation whereby new blood cells carry the corrected or modified genes. Engraftment is closely followed up because effective engraftment forms the basis for the therapeutic effects of the treatment. [12][17]

Engraftment Kinetics: This refers to the time of maturity in which the edited cells start to produce mature blood cells. Engraftment usually starts within weeks, but it may take months for full recovery in haematology. Success of this process tends to represent the efficacy of the treatment and is largely monitored continuously through periodical blood tests and bone marrow biopsies [15] .

6. Monitoring after Transplantation and Outcomes

Monitoring: The patients are vigorously and continuously monitored for the success of engraftment and, in turn, for the long-term effects of gene-editing therapy after transplantation. This would involve regular blood tests to check for the appearance of normal blood cells, imaging studies to monitor the health of a patient's bone marrow, or probably biopsies to prove the stability and functionality of the transplanted cells. Monitoring for adverse effects, such as graft-versus-host disease or GVHD, and signs of malignant transformation, is imperative as well [17].

Long-Term Efficacy: Clinical trials and follow-up studies proved that HSCs edited by CRISPR-Cas9 have durable and curative therapeutic effects in patients with genetic blood disorders, including sickle cell disease and beta-thalassemia. It has been evidenced that this treatment considerably minimizes or clears up the symptoms of the above diseases, hence resulting in improved quality of life and, in many cases, long-term remission. [14][17]

Increased Foetal Haemoglobin (HbF)

Role of HbF

1.HbF Function in Reducing the Sickling Capability of Red Blood Cells.

In sickle cell disease, it is the mutant HbS that causes red blood cells' propensity to sickle, leading to vaso-occlusion and serious morbidity. Because the structure of HbF is different from that of HbS, HbF inhibits the polymerization of HbS and is protective against sickle cell disease. Preclusion of this process will avoid red cells from undergoing sickling and hence reduce vasoocclusive events, pain, and organ damage. It basically maintains RBC deformability, hence preserving the HbF role in the symptomatic and complication management of SCD.[18]

2.HbF Mechanisms in SCD Management:

As observed earlier, this is instrumental in the management of SCD by its reduction of HbS in the red blood cells. The more the level of HbF, the more the space in the red blood cell that is occupied, and hence the less for HbS. Such dilution lowers the chances that HbS will polymerize in conditions of low oxygen, a critical step in the principal mechanism leading to

cell sickling. Thus, higher levels of HbF are directly related to a reduced frequency of sickle cell crises, which ultimately translates to better health for SCD patients.[19]

3. Therapeutic Strategies to Increase HbF

The raising of HbF levels in SCD patients has, therefore, gone through an evolutionary path in therapies. Classical stimulations treatments are mainly hydroxyurea, and most cases use it, as it stimulates the HbF production. More recent intervention, CRISPR-Cas9, is a much more targeted one by manipulation of BCL11A, a gene that would otherwise repress the production of HbF. By editing such genes, CRISPR-Cas9 allows sustainable production of HbF and is therefore viewed as a potential cure for SCD. These developments emphasize the importance of HbF in SCD symptom management and possibly the disease cure as well. [17]

CRISPR's Impact

Specifically, CRISPR-Cas9 editing disrupted the BCL11A gene, a known foetal haemoglobin (HbF) repressor in adult erythrocytes. In the absence of HbF expression, the BCL11A protein causes repression by binding to specific DNA regions. Knockout or modification of the BCL11A gene with CRISPR-Cas9 relieves this suppression, leading to an increase in HbF production. This is because the higher HbF dilutes the HbS, reducing its polymerization and preventing RBC sickling, thereby ameliorating symptoms of SCD.[12][13][17]

- **Mechanism of Targeting** BCL11A in Hb by CRISPR-Cas9 the CRISPR-Cas9 system is composed of the Cas9 protein and guide RNA that targets the BCL11A gene in hematopoietic stem cells. It is the guided RNA that binds itself at a specific position to the BCL11A gene, guiding the Cas9 protein to the spot.

- **DSB Induction:** Cas9 induces a double-strand break upon locating at the target locus. Such a break can then be repaired through HDR by the cell, which is programmable to produce site-directed changes when an appropriate template is added, or by Non-Homologous End Joining, often a mutagenic process.

- **BCL11A inactivation:** Since NHEJ is known to cause small insertions or deletions during the time of break repair, it can be assumed that inactivation of BCL11A is one such outcome of rupture events. When the rupture leads to knockout of BCL11A, this breakdown releases the repressive impact on the HbF gene, allowing for resumed HbF synthesis.

- **Increased HbF Levels:** The reactivated HbF replaces some of the HbS within the red blood cells; unlike HbS, which polymerizes, HbF does not add to the pathognomonic red blood cell sickling of sickle cell disease.

- **Therapeutic Effect:** Reduced haemolysis and red blood cell sickling associated with increased HbF levels decrease manifestations of sickle cell disease, improving the prognosis of patients.

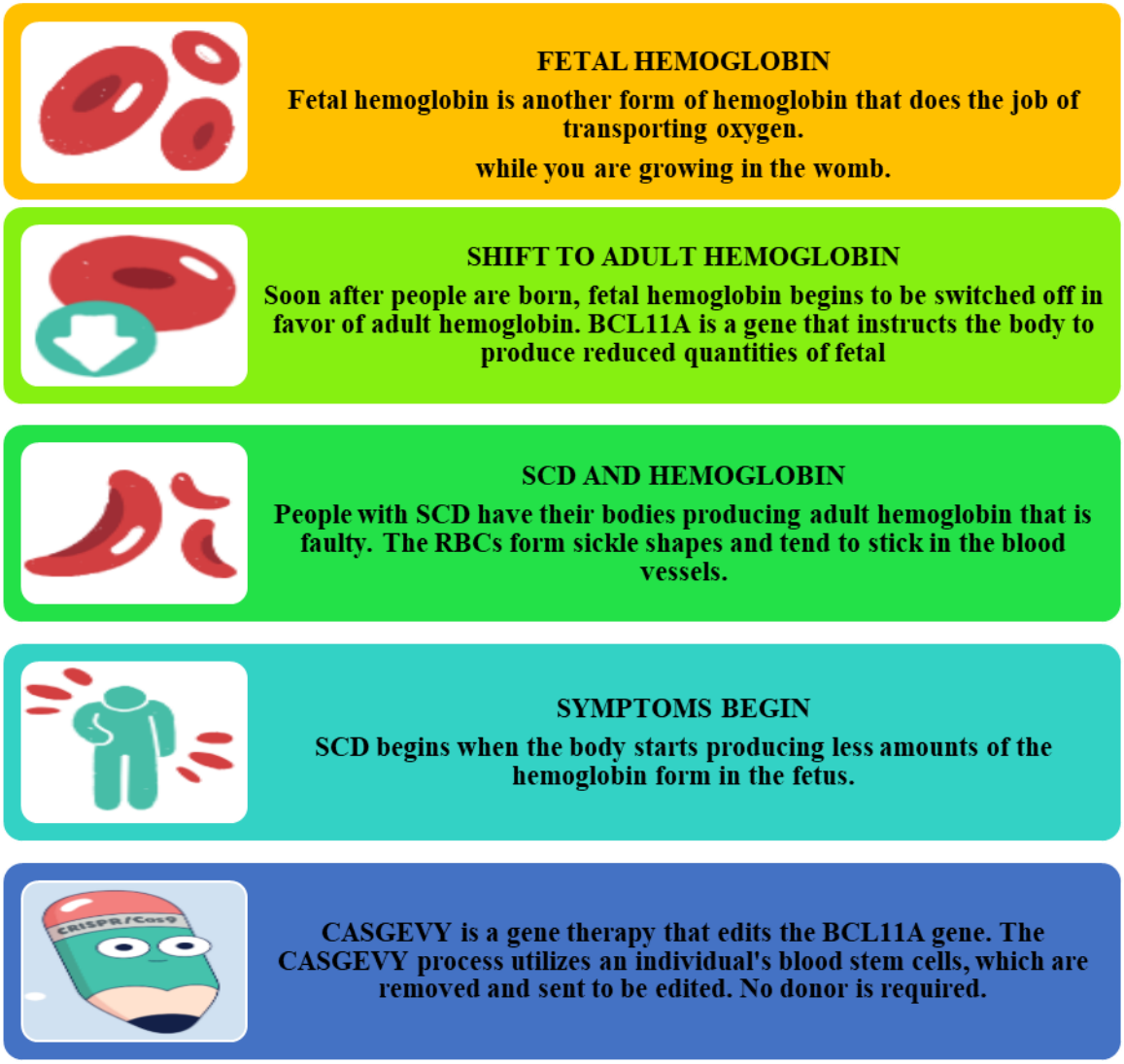
CLINICAL IMPLICATIONS

In fact, the clinical trials that proved high promise for this approach did reveal quite large benefits to SCD patients: high haemoglobin related to a low degree of sickness severity, less frequent pain crises, and generally improved quality of life for the patient.

CASGEVY: THE FIRST CRISPR THERAPY

Patients 12 years of age and older with recurrent vaso-occlusive crises or vaso-occlusive events may receive treatment for sickle cell disease using Casgevy, a cell-based gene therapy. With respect to genome editing, CRISPR/Cas9 is being used in the first FDA-approved therapy: Casgevy. Genome editing with CRISPR/Cas9 technology is performed on hematopoietic (blood) stem cells from patients.[19]

Before learning about how CASGEVY works, let's learn about the role that foetal haemoglobin plays in sickle cell disease (SCD).

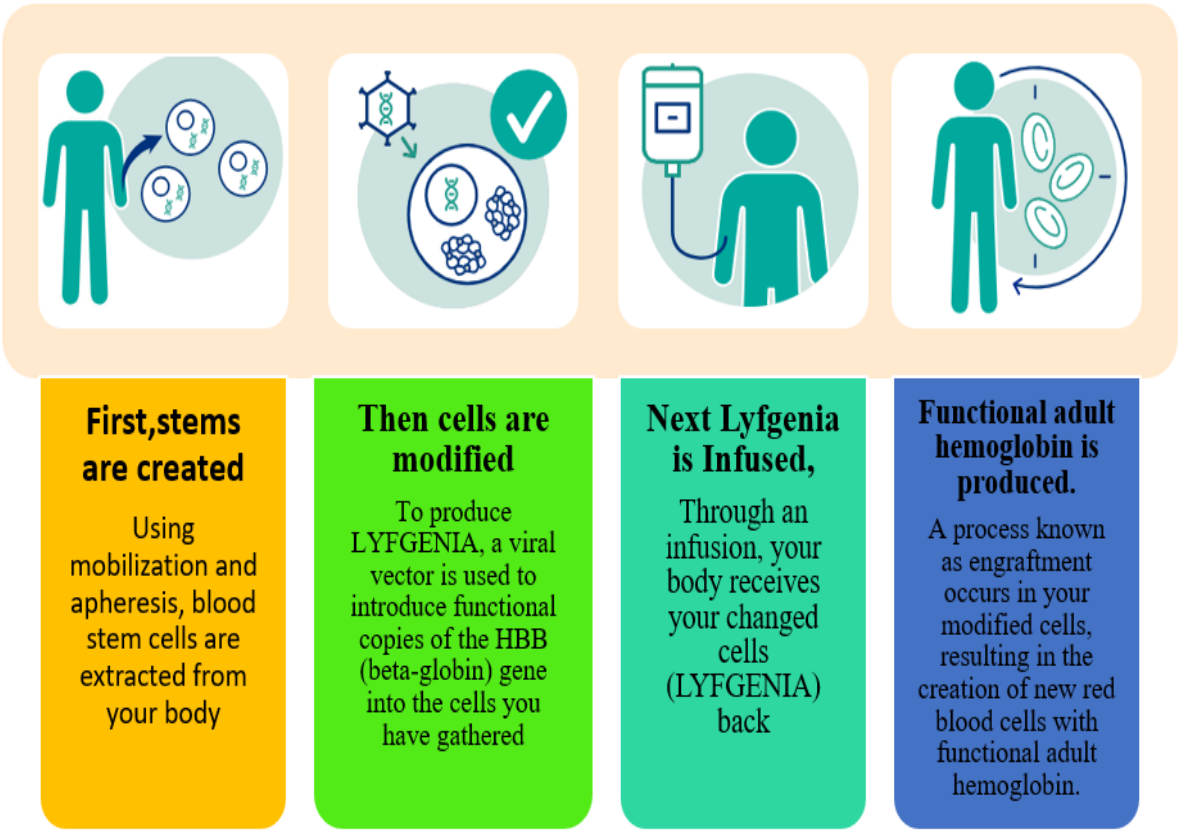


CASGEVY working [20]

Targeted DNA editing is made possible by the ability of CRISPR/Cas9 to precisely edit (remove, add, or replace) DNA where it has been cut. The altered blood stem cells are then re-infused into the patient, where they engraft (attach and multiply) within the bone marrow and increase the production of foetal haemoglobin (HbF), a type of haemoglobin that helps carry oxygen; in sickle cell disease patients, elevated HbF levels prevent red blood cell sickling.[21]

Implications and Benefits of Casgevy

Casgevy stands out as a ground-breaking intervention, marking the first gene therapy for Sickle Cell Disease (SCD). Unlike traditional treatments, Casgevy offers the potential for a curative approach, providing hope for recovery to a significant number of patients. A key advantage of this therapy is its ability to eliminate the need for repeated blood transfusions and transplant procedures, thereby greatly improving the quality of life for individuals with SCD. Additionally, Casgevy reduces the risk of graft-versus-host disease and lowers the dependency on immunosuppressive medications, making it a safer option compared to conventional stem cell transplant methods. Beyond its safety benefits, the therapy also shows promise in reducing vaso-occlusive events, a common and severe complication of SCD. This wide-ranging impact positions Casgevy as a transformative treatment, offering a safer and more accessible alternative to the current range of SCD therapies. In summary, while existing FDA-approved treatments for SCD address specific aspects of the disease, they do not provide a comprehensive, stand-alone solution. Casgevy, with its exceptional safety profile, curative potential, and ability to address disease complications, emerges as a promising revolutionary treatment for SCD. [22]



How Does LYFGENIATM (lovotibeglogene autotemcel) works[26]

CRISPR CLINICAL DATA FOR CASGEVY

The UK's Medicines and Healthcare Products Regulatory Agency authorized Casgevy on November 16, 2023, for the treatment of SCD and Transfusion-dependent beta thalassemia (TDT) in patients who are 12 years of age or older. On December 8, 2023, the US Food and Drug Administration (FDA) subsequently approved SCD. These were the first CRISPR-based treatment approvals in history. Since then, Casgevy has received conditional approval

in Bahrain, approval in the EU, and approval in the US for the treatment of TDT. The Saudi FDA is now reviewing a regulatory proposal, with a Canadian filing anticipated in 2024. [23] Data Supporting Casgevy. These approvals are the result of phase 3 trial data including adults and children with severe sickle cell disease. Vertex and CRISPR Therapeutics have shared data from 17 SCD patients: The effects are striking and long-lasting. After receiving therapy, 16 out of 17 SCD patients no longer experience the vaso-occlusive crises that are typical of the condition. There have been no hospitalizations for vaso-occlusive crises involving the other patient. [24]

LYFGENIA: ANOTHER CELL BASED GENE THERAPY

Lyfgenia is a cell-based gene therapy. Lyfgenia, which uses a lentiviral vector (gene delivery vehicle) for genetic alteration, is approved for the treatment of sickle cell disease patients aged 12 and up who have a history of vaso-occlusive episodes. Lyfgenia involves genetically modifying the patient's blood stem cells to create HbAT87Q, a gene-therapy derived haemoglobin that functions similarly to haemoglobin A, the normal adult haemoglobin produced in people who do not have sickle cell disease. Red blood cells with HbAT87Q had a decreased risk of sickling and obstructing blood flow. The transformed stem cells are subsequently administered to the patient. [25]

CLINICAL DATA SUPPORTING LYFGENIA

The safety and effectiveness of Lyfgenia is based on the analysis of data from a single-arm, 24-month multicenter study in patients with sickle cell disease and history of VOEs between the ages of 12- and 50- years old. Effectiveness was evaluated based on complete resolution of VOEs (VOE-CR) between 6 and 18 months after infusion with Lyfgenia. Twenty-eight (88%) of 32 patients achieved VOE-CR during this time period.

CONCLUSION

The most astounding, vastly target-oriented revolutionary treatment of sickle cell anemia is the CRISPR-Cas9 technology. It tries to correct the basic gene abnormalities leading to the disease. With this new tool of genome editing technology, one can treat the cause of the disease at its very roots through the correction of haemoglobin genes which are faulty. It gives new hope both to the possibility of a single curative therapy and alleviation from the debilitating symptoms due to sickle cell anemia. However, there are major obstacles blocking the way toward clinical application: delivery methods, potential off-target consequences, and the ethical plane. It, however, awaits studies and clinical trials to really optimize the technology to be both safe for wide-scale application and efficacious. Studies being conducted on this gene-editing tool, CRISPR-Cas9, can sometime later be translated into the quick changing of tides in this therapy from management of symptoms to a curative strategy for sickle cell anemia, opening up a totally new prospect in personalized medicine and targeted drug delivery.

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