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A Review Article On Gene Editing By CRISPR-Cas9 For The Treatment Of Sickle Cell Anemia

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Abstract

Sickle cell disease (SCD) is a congenital blood illness caused by faulty haemoglobin synthesis, resulting in sickle-shaped red blood cells. This condition is caused by a single point mutation in the beta globin gene. SCD is primarily caused by a mutation in the gene that produces haemoglobin, the protein that transports oxygen in red blood cells. SCD patients may have chronic pain, exhaustion, anemia, stroke, organ damage, and increased infection risk. SCD treatment options focus on symptom management and preventing complications. Treatment options include supportive care, pharmacological therapies, hematopoietic stem cell transplantation, gene therapy, and gene editing. Gene editing can accurately fix inherited blood diseases like SCD.SCD focuses on symptom management and preventing complications. This covers supportive care, pharmacological therapies, hematopoietic stem cell transplantation, gene therapy, and gene editing. Gene editing is a promising treatment for genetic blood disorders like SCD. It can remove harmful variations, alleviate symptoms, and even cure the condition altogether. The CRISPR-Cas9 gene editing method is utilized to cure SCD.

Keywords: sickle cell illness, stroke, hematopoietic stem cell transplantation, gene editing, CRISPR-Cas9.

Introduction

Sickle cell disease (SCD) is a congenital blood illness where faulty hemoglobin molecules lead red blood cells to form a crescent or sickle shape. This illness affects millions of people globally, especially those of African, Mediterranean, Middle Eastern, and South Asian heritage. SCD has a substantial impact on the health and well-being of those affected. Understanding the reasons, symptoms, and treatment choices is crucial for healthcare professionals, patients, and family members.

SCD is mostly caused by a mutation in the beta-globin gene (HBB), which produces hemoglobin.This gene mutation results in hemoglobin S (HbS), an aberrant haemoglobin with a unique molecular structure compared to normal adult HbA. HbS stiffens red blood cells, reducing their ability to flow via tiny capillaries. SCD-related health issues stem from altered red blood cell shape. Treatment for SCD aims to manage symptoms, prevent complications, and enhance patients' quality of life. Effective pain management often requires the use of analgesics such as opioids, nonsteroidal anti-inflammatory medications, and adjuvant therapy. Adequate hydration, both orally and intravenously, is crucial for maintaining healthy hydration, both oral and intravenously, is crucial for maintaining healthy blood flow. Blood transfusions may be necessary to enhance oxygen supply to tissues and lower the risk of consequences like stroke.

Healthcare practitioners must understand the causes, symptoms, and treatment options for SCD to provide accurate diagnosis, effective symptom management, and counseling to patients and families. Advancements in gene therapy may lead to better treatments for SCD patients.

Types of Sickle Cell Disease

1. Sickle cell anemia (SCA) is the most severe variant, defined by homozygous HbS (HbSS). 2. Sickle haemoglobin C disease [HbSC] is a milder variant defined by compound heterozygous HbS and HbC.

3. Sickle beta thalassemia [HbS / beta -thalassemia] is a variable severity condition with a combination of HbS and beta-thalassemia.[17-19]

Causes

1. autosomal recessive inheritance

2. Mutation in HBB gene.

3. Homozygous Mutation

4. Family History

5. Reduced oxygen levels

6. Dehydration

7. Genetic Variation [17–19]

Symptoms:

SCD is characterized by a wide range of symptoms that can vary in severity and presentation among individuals. The symptoms primarily arise due to the abnormal sickling of red blood cells and subsequent complications. This section provides a detailed overview of the common symptoms associated with SCD. [8-11]

1.Pain crises
2.Anemia
3.Infection
4.Acute chest pain
5.Delayed growth and development
6.Stroke
7.Organ damage

Diagnosis

The diagnosis of SCD involves a combination of clinical evaluation, laboratory tests, and genetic testing. The aim is to identify the presence of abnormal HbS and assess the extent of the disease. This section provides a detailed overview of the investigations and diagnostic approaches used for SCD.

Complete blood count-A total blood count helps assess the levels of hemoglobin, red blood cells, and other cell types. Due to chronic hemolysis, individuals with SCD typically exhibit a lower hemoglobin level and a higher reticulocyte count.

Sickledex/solubility test –It is also known as the sodium metabisulfite test or solubility test. It is a quick screening test that detects the presence of HbS in a blood sample. It relies on the insolubility of HbS under certain conditions, leading to the formation of sickle-shaped cells. However, this test is less specific than haemoglobin electrophoresis and may require confirmation with additional tests.

Haemoglobin electrophoresis- It is a laboratory test that separates and identifies different types of haemoglobin in blood based on its electrical charge and size.

Haemoglobin high-performance liquid chromatography - Separates and quantifies haemoglobin variants, including HbS , HbA ,HbF and HbA2 and identifies abnormal haemoglobin patterns characteristic of SCA.

Genetic testing- Genetic testing for sickle cell anemia involves analysing the HBB gene to identify mutations that cause the disease.

Newborn screening – newborn screening for sickle cell anemia is vital public health program that enables early detection ,intervention and management. [1,7,12,13,14,15,16]

Sickle cell disease (SCD) mechanisms and pathophysiology:

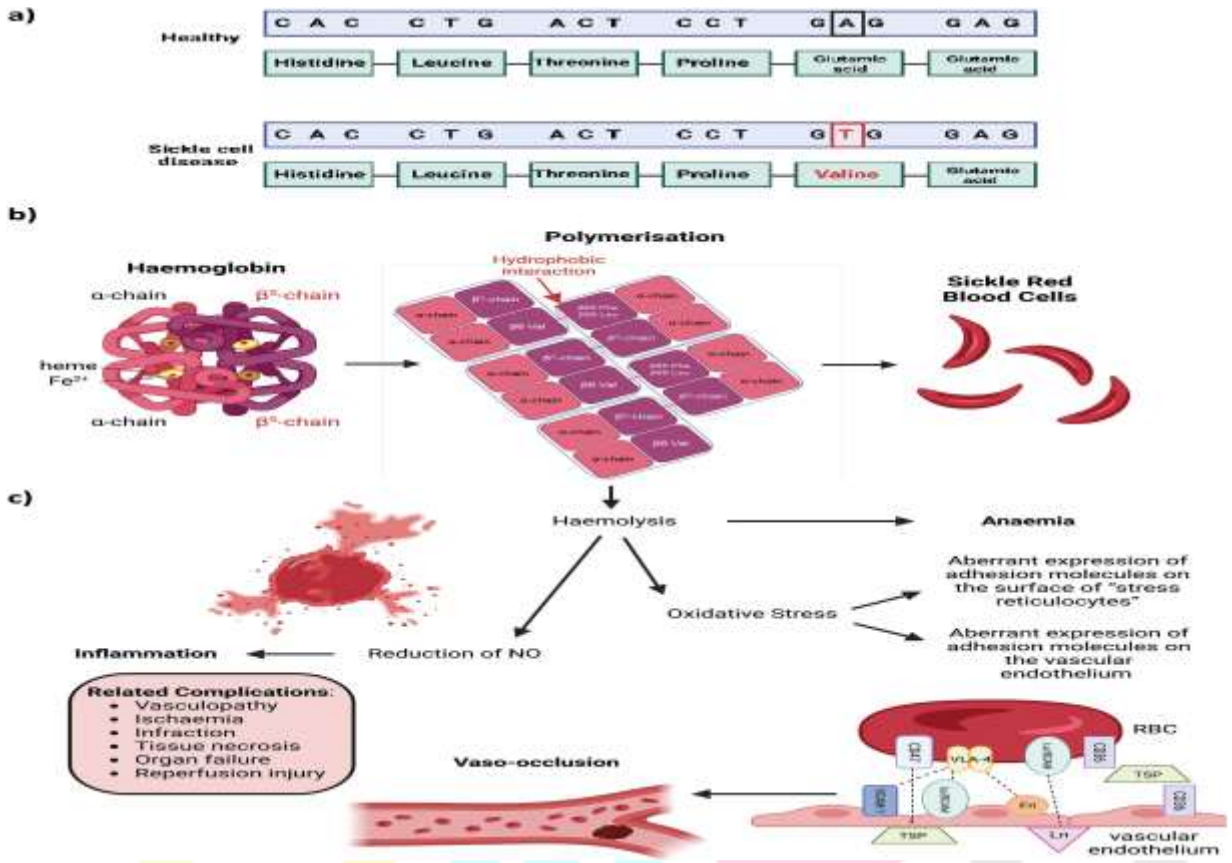

Fig 1: a) Schematic diagram of the DNA and amino acid sequences for normal and SCD individuals. A homozygous point mutation (GAG > GTG) is present in the HBB gene, which substitutes glutamic acid to valine .

b) The basis of SCD pathophysiology. (Left) Haemoglobin is made of 2 α- and 2 β-chains, each containing a heme molecule, used to bind Fe2+ ferrous ion. (Middle) The amino acid change (Glu to Val) caused by SCD-causing mutation causes haemoglobin proteins to create hydrophobic interactions during polymerisation under deoxygenated conditions. (Right) The result is the formation of sickle red blood cells.

c) Pathophysiological features of sickle cell disease. Sickle haemoglobin polymerisation causes a cascade of events: haemolysis directly causes anaemia, while oxidative stress, related to the release of free heme and ions, causes the aberrant expression of adhesion molecules (Fn - fibronectin; TSP – thrombospondin, Ln - laminin, VLA-4 or α4β1 integrin, Lu/BCAM - basal cell adhesion molecule or Lutheran blood group) on the surface of "stress reticulocytes", a population of young RBCs coming out of the bone marrow prematurely, and on the vascular endothelium, causing vaso-occlusion. Finally, hemolysis causes the reduction of Nitric Oxide (NO), resulting in inflammation.

Treatment:

1. Supportive care:

Pain Management: SCD's acute pain crises are treated with analgesics including nonsteroidal anti-inflammatory medicines, opioids, and patient-controlled analgesia. Non-pharmacological treatments may include heat therapy, relaxation, and distraction strategies. [20]

Hydration: Staying hydrated helps to prevent vaso-occlusive crises. Patients should stay hydrated, especially during high-risk situations like illnesses or excessive temperatures.

Blood transfusion: Red blood cell transfusions may be done for severe anemia, acute chest syndrome (ACA), or stroke. Transfusions improve blood oxygenation and lower sickle cell count.[21]

2. Supportive measures:

Comprehensive care programs include frequent medical follow-up, psychosocial support, educational resources, and genetic counselling to individuals with SCD. These interventions improve disease management and quality of life for individuals and families [22]. To prevent pain crises, patients with SCD should be educated about triggers, hydration, and early symptom recognition. To prevent illness, it's crucial to treat underlying infections promptly and avoid severe temperatures [23]. Individuals with SCD require regular medical treatment from experienced clinicians who understand the illness. Individualized treatment strategies should consider illness severity, potential consequences, and patient needs.

3. Drug therapy:

Although we have a better understanding of SCD pathogenesis, present therapies mostly address symptoms. This includes preventing HbS polymerization and minimizing its consequences. [24]

Hydroxyurea:

Hydroxyurea (or hydroxycarbamide) is an FDA-approved HbF inducer for the treatment of SCD [25]. Despite decades of research, the specific mechanism by which hydroxyurea stimulates HbF synthesis is unknown. Clinical trials and in vivo investigations reveal a more complex mechanism. Hydroxyurea inhibits ribonucleotide reductase, an enzyme involved in DNA synthesis, leading to cell cycle arrest and cell death. This promotes recruitment of erythroid progenitor cells and HbF synthesis [26]. It also disrupts repressor-cofactor connections at globin promoters and stabilizes activator interactions [27]. Hydroxyurea has been linked to fewer neutrophils and reticulocytes in SCD patients, leading to lower morbidity and death [28,29]. It promotes erythrocyte hydration and deformability. It releases nitric oxide (NO) to combat NO deficiency caused by hemolysis.[31,32] It is generally considered safe for both adults and children, with response rates of up to 70%.[29,30,27] However, its effectiveness varies widely from patient to patient for unknown reasons.Non-adherence to regular dosing or inadequate dosing may be the most likely reasons for lack of response [33,34]Hydroxyurea is considered the bestdisease-modifying therapy for sickle cell disease, reducing symptoms,mortality, and global availability. [29]

Crizanlizumab:

Crizanlizumab is an FDA-approved monoclonal anti-P-selectin antibody for SCD that reduces vaso-occlusive events by inhibiting P-selectin on the vascular endothelium. This limits the ability of activated neutrophils to capture sickle cells, preventing the cascade of events that precedes a vaso-occlusive event [35,36]. As the first FDA-approved therapy for SCD since hydroxyurea, it addresses cell adherence, a problem that hydroxyurea does not tackle [37]. Consequently, the European Commission withdrew its marketing authorisation in 2023, a decision influenced by the STAND trial results, which showed no statistically significant difference in pain crisis rates between the treatment and placebo groups in the first year. [38]

Voxelotor:

Voxelotor (GBT440 or Oxbryta) is an oral medication designed to increase the oxygen affinity of haemoglobin, stabilize its oxyhemoglobin state, reduce deoxyhemoglobin HbS concentration, and decrease polymerization of sickle cells.[35-39] Furthermore, it increases oxygen availability and stabilizes the nonpolymerized relaxed state of haemoglobin , thus reducing disease severity.[40,42] Early clinical trials showed that it improved haematological parameters with minimal side effects.[42] While Phase 3 trials confirmed that it was effective in increasing haemoglobin concentrations, reducing anemia, and preventing hemolysis, with a favourable safety profile.[43] Voxelotor is contraindicated in individuals with a history of drug hypersensitivity or when using CYP3A4 inhibitors, and may interfere with the identification of Hb subtypes by high-performance liquid chromatography.[44]

L-glutamine:

L-glutamine, another drug approved by FDA to reduce SCD frequency. Complications- In essential amino acids that may function through production Nicotine amide adeninju nukureotides in red blood cells, potentially reduced oxidative stress, Prevention of crisis related to sickle.[35,45,46] However, the important field is not displayed in the phase 3 test. Changes in the number of haemoglobin, hematical level, or mesh red blood cells despite the decrease in the pain crisis [46] Field Despite FDA approval, its efficacy and safety are less established than for hydroxyurea, and there is limited patient accessibility due to high cost.[45,47]

Combination drug therapy:

Combination drug therapy is a recently developed strategy in which various therapeutic agents are mainly anti-interferon and anti-sickle cell drugs combined [41]. This approach is the result of a deep understanding of the polymerization process of deoxygenated HbS. and hemoglobin switching, the proof-of-concept of which was confirmed in a study by Atweh et al. Studies combining hydroxyurea and butyrate [48] . Combination treatment regimens need to be defined. To allow this strategy to be implemented in clinical practice. It will likely be designed with gravity in mind. Extensive testing in clinical phenotypes and animal models will be required, ultimately leading to the development of new approaches to precision medicine.[41]

Benefits is to improved symptom management, reduced disease complications, enhanced quality of life and potential for increased survival.

1. Hydroxyurea + Folic Acid: Enhances fetal haemoglobin production.
2. Hydroxyurea + Erythropoietin: Stimulates erythropoiesis.
3. Penicillin + Vaccination: Prevents infection.

4. Hematopoietic stem cell transplantation:

Current treatment strategies for sickle cell disease are primarily aimed at palliation of symptoms, allgeneic hematopoietic stem cell transplantation(HSCT).Her has been proposed mainly for pediatric patients before development of irreversible vascular lesion [49]. Studies have shown very good result: overall survival >93% and event –free survival >86%[50,51,52]. The biggest drawbacks of this approach are the need for myeloablatives conditional , infection ,and grafts-versus-host disease (GVHD) in 12-14% of cases [53,49,54].furthermore, search for antigen (HLA) of human leukocytes the appropriate donor of brother and sister who solve the success of the transplant is a problem of 80% or more . SCD patient [61] transplantation of navel blood on the ground provide less alternative. Although it is a strict comparison of HLA, the risk of GVHD is high, transplantation related mortality rate. [55,56]

A recent advance in sickle cell transplantation is in utero stem cell transplantation(IUSCT), which aims to treat sickle cell disease before birth using immature stem cells. The fetal immune system. However, it faces challenges such as differing engraftment rates. Innate and allogeneic cells and maternal immune responses.[57-58] Moreover,the main limitation of this approach is competition with host cells, which makes it difficult for donor cells to compete with the rapidly growing and developing embryonic compartment. Long-term chimerism has

been successfully maintained [57,59,60]. When using IUSCT, compared to other transplantation methods, ethical and safety consideration must be taken into account.

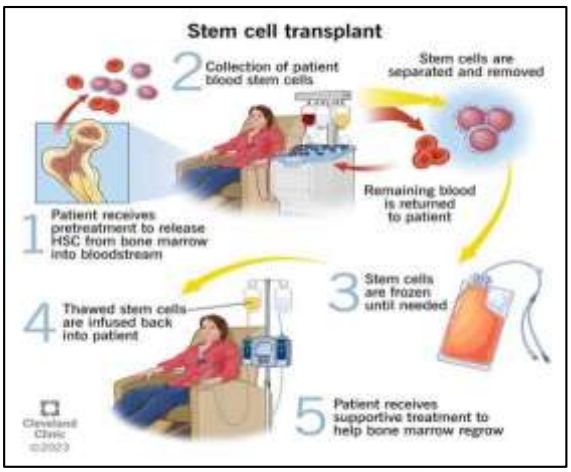

Fig 2 : Hematopoietic stem cell

5. Gene Therapy:

SCD gene therapy is a rapid development area motivated by the design of Vectors specific to fabrics and efficient and gene editing technologies. Initial approaches Adding β or γ-globin genes involved, while more recent strategies aim to reactivate HBF Expression, obtaining results similar to hydroxyurée [62]. Successful gene therapy β-thalassemia has encouraged numerous clinical trials in sickle cell disease using two main approaches: lentiviral vectors carrying β- or γ-globin genes and BCL11A, thereby inducing endogenous HbF [63].

Clinical trial of vector-based gene therapy (LentiGlobin BB305) Lentivirus delivering a modified β-globin gene (Thr87Gln substitution) shows excellent potential. This This approach includes CD34 +hematopoietic rods and the modification of the front body cells separated by patients.(HSPCS) fixed adult haemoglobin aimed at hindering Hbat87Q candeled haemoglobin polymerization [64] this domain was first approved by FDA foβ-β- r in august 2022. Reduced effect such as zynteglo (beteglogen autotemcel or beti-cell) the blood transfusion needs in β-tallasemy have been established [65,66,67] field bluebird bio recently obtained a second approval for Lyfgenia (lovotibeglogene autotemcel), based on the same lentiviral vector and gene payload and differing from zynteglo in small manufacturing feature [68].

For SCD, recent Bluebird Bio trials have shown encouraging results, including corrected haematological parameters and reduced vaso-occlusive crises 15 months after treatment[69]. Furthermore, advancements in gene editing technologies offer new therapeutic avenues for hemoglobinopathies, aiming to specifically modify patients genetic material to correct disease-related genes or act on the disease mechanism at a molecular level[70]. These gene editing strategies, aiming to correct the β-globin gene mutation or induce fetal γ-globin production, could provide a one-time cure for SCD, highlighting the dynamic evolution of gene therapies in treating haematological disorders[71].

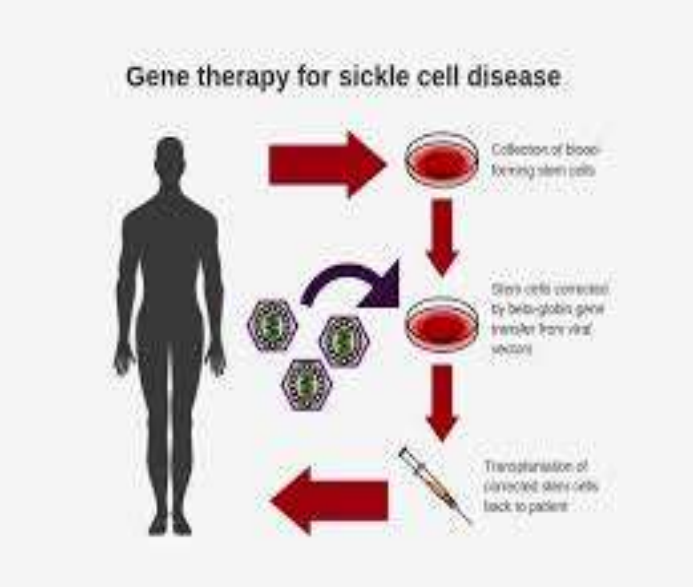

Fig 3: Gene therapy SCD

Gene Editing:

Gene editing for genetic blood disorders such as SCD is a promising treatment option, resulting in precise correction of pathogenic variants, improvement of disease symptoms, or even complete cure of the disease [70] . Gene editing technologies are based on targeted nucleases that have been developed and refined over many years.

The first nucleases used for gene editing are meganucleases, zinc finger nucleases, and transcription activator-like effector nucleases (TALENs). Meganucleases, or localization endonucleases, are natural monomers that recognize relatively long sequences (14–40 bp) involved in DNA repair [72]. The low frequency of meganuclease target sites in the human genome limits their use as therapeutics; however, target recognition can be engineered by structure-guided design and high-throughput screening [70,72]. Another limitation is their reduced specificity caused by sequence degeneracy. On the other hand, zinc finger nucleases (ZFNs) are chimeric nucleases consisting of zinc finger protein (ZFP) motifs, the most common DNA binding motif in humans, each of which can recognize short DNA sequences (3–4 bp).Therefore, they have been assembled to enhance their DNA recognition ability [70,73] .The main advantages of this technology are motif engineering by site-directed mutagenesis and broad targeting ability achieved by rational design or selection of combinatorial libraries [74]. As a result, ZFNs are generated by fusing a zinc finger protein DNA binding domain with the endonuclease FokI [75]. Cleavage activity is induced only when two ZFNs recognize the target sequence, usually separated by a 5–7 bp spacer region, and form a catalytically active heterodimeric complex [75,70,76]. TALENs are another family of chimeric nucleases with a DNA-binding domain and a FokI nuclease. The DNA-binding domain differs from the ZFNs, consisting of an array of TALE repeats and 34 amino acid proteins whose residues 12 and 13 determine DNA specificity [77,74] . Specificity is again controlled by a heterodimerization event that catalytically activates the FokI nuclease of the TALEN DNA-binding monomer pair. Tuning target specificity in this case is easier than with ZFNs, since the TALEs are repeated. In theory, any sequence can be combined to target [78]. Further progress has been made with the development of hybrid mega-TAL nucleases consisting of easily accessible TALE DNA-binding domains, modifiable and site-specific heads of meganucleases that are active as monomers. They are characterized by their high specificity, affinity, and intracellular delivery due to their small size (approximately 75 kDa) [79].

Gene Editing Techniques:

1. CRISPR-Cas9: Uses RNA-guided DNA endonuclease to edit genes.
2. CRISPR-Cpf1: Similar to CRISPR-Cas9, but with different enzyme.
3. TALENs (Transcription Activator-like Effector Nucleases): Uses DNA-binding domains to edit genes.

4. ZFNs (Zinc Finger Nucleases): Uses zinc finger proteins to edit genes.
5. Base Editing: Directly edits individual DNA bases without cutting DNA [80,81,82] .

CRISPR-Cas9 Editing:

CRISPR (cluster regularly connected to short palindromic repetitions) is a recent Breakthrough in the field of genetics, a revolution to revolutionize the way scientists approach gene processing and molecular therapy. Initially observed as a peculiar repetitive scheme in bacteria DNA, was subsequently understood as a form of immune time against viruses [83]. The crispr the system is essentially a natural tool that uses bacteria to protect themselves "remembering" parts from the penetration of the viral DNA and therefore of targeting when it enters bacteria a second time [84]. This discovery has been adapted for use in genetic manipulation, which means that targeted changes are possible Different organisms with high precision. The ease of use of the Crispr, precision system Efficiency has made a crucial tool in modern genetic research, which means that the development of New genical therapies [85].

The CRISPR-Cas system, first discovered in 1987, serves as an adaptive immune system present in approximately 40% of bacteria and 90% of archaea [86,87]. This system plays a crucial role in creating cellular memory that provides protection against foreign organisms [88].It consists of a CRISPR locus that includes short repetitive elements known as repeats, separated by unique sequences called spacers.

CRISPR-Cas9 Components:

1. Cas9 (CRISPR-associated protein 9): Endonucleasw enzyme.
2. Guide RNA (gRNA): 20-24 nucleotide RNA sequence.
3. Protospacer adjacent motif (PAM): 3-nucleotide DNA sequence.

CRISPR-Cas9 mechanism:

Step 1: Target Recognition

1. gRNA binds to Cas9 enzyme .
2. gRNA recongnizes target DNA .
3. PAM sequence adjacent to target DNA is recognized.

Step 2: DNA Cleavage

1. Cas9 enzyme cleaves target DNA at specific location.
2. Double-stranded break (DSB) is generated.

Step 3: Repair Machinery Activation

1. Cells repair machinery is activated.
2. DNA repair pathways (NHEJ or HDR) are triggered.

Step 4: Gene Editing

1. NHEJ (Non-Homologous End Joining): Error-prone repair.
2. HDR (Homologous Directed Repair): Precise editing with template.

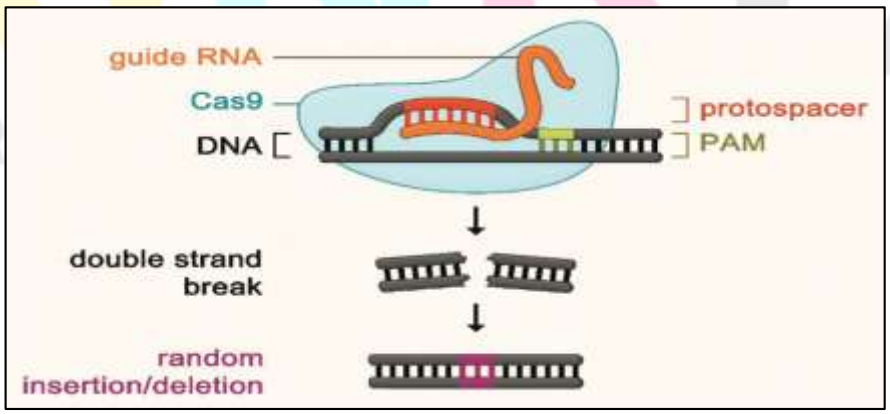

Fig 4 : The genome-editing mechanism of regularly clustered short palindromic repeats (CRISPR)/CRISPR-associated (Cas) is outlined.

These CRISPR repeat-spacer arrays undergo transcription to produce CRISPR RNA (crRNA) and transactivating CRISPR RNA (tracrRNA) [89,90]. In addition, the system includes a set of CRISPR-associated

(cas) genes that encode Cas proteins. These proteins play a role in flanking the A-T-rich leader sequence preceding the CRISPR array [91]. The typical mechanism of action for the CRISPR-Cas system involves modification or integration of spacers, biogenesis of crRNA, and interference of DNA or RNA [92]. CRISPR-Cas systems are classified into two major classes based on their Cas gene structure. Class 1, includes Types I, III and IV work with multiple CAS proteins and is more complex. On the other hand, Class 2, including types II, V and VI, is mainly based on a single single CAS protein for his function, making it structurally simpler [93]. In these classes, the CRISPR-CAS9 system (Type IIA) is more commonly used when generating genes. It consists of two main components: single Leiden RNA (Sgrna) and Cas9 protein [94]. The group consists of two components of the EN: Crna and Tracrna, which, when they are dissolved, can focus on almost all gender investigations. The Cas9 protein is a 160 kDa DNA endonuclease responsible for cleavage of target DNA and formation of a double-strand break. It has two lobes: the recognition lobe (REC) which is involved in binding sgRNA and the nuclease lobe (NUC), which plays a role in DNA cleavage and conferring specificity for the protospacer adjacent motif (PAM), a short (2–6 bp) DNA sequence found downstream of the target sequence [95]. In the original bacterial Cas9 system (S. pyogenes Cas9 or SpCas9) the PAM sequence plays a crucial role, allowing specific recognition of viral DNA integrated into the host bacterial genome, characterized by a PAM sequence NGG, which allows distinguishing viral DNA from the CRISPR locus [94].

The Cas12a protein (also known as Cpf1), a component of the type V-A CRISPR-Cas9 systems, offers unique features that differ from the better known Cas9 [96]. Unlike Cas9, Cas12a can process its own crRNA due to its RNase site, eliminating the need for tracrRNA. This makes Cas12a a dual-function protein with both endoribonuclease and endonuclease activities [97]. A significant aspect of Cas12a is its recognition of specific PAM sequences (TTTV) and its ability to create staggered DNA breaks downstream of these sites. These breaks result in overhanging ends, which are advantageous for precise gene insertion[98]. Cas12a is noted for its enhanced activity, greater specificity, and reduced off-target effects compared to Cas9 [99]. More recently, a novel base editor Cas12m GoCas12 has been developed, which has no DNA cleavage activity, thus increasing the likelihood of DSBs and large genomic rearrangements are minimized [100]. However, the choice between Cas12a and Cas9 must be based on the specific requirements of the gene editing task, as each system has its strengths and applications[98].

The CRISPR-Cas9 system has been used to treat SCD using two strategies: i)correcting the mutation to the wild-type allele so that cells produce higher levels of expressing HbA, thereby rescuing the SCD phenotype; and ii) inducing HbF production by disrupting gene regulatory elements, thereby reducing sickle cell disease and improving quality of life. A 2016 study used Cas9 ribonucleoproteins (RNPs) consisting of Cas9 protein and a single HDR donor guide and template RNA delivered by adeno-associated viral (AAV) vectors in CD34+ HSPCs, achieving promising HDR-mediated gene editing frequencies at the HBB locus [101]. Similarly, other studies have investigated ex vivo correction of SCD mutation in CD34+ HSPCs have been shown to exhibit variable efficiencies ranging from 18% (Hoban, Lumaquin et al. 2016), 25%, 60% , 64% and even 94% .

In recent years, emphasis has been on inducing high levels of HbF in SCD patientsby either regulating transcriptional repressors or generating hereditary persistence of fetal haemoglobin (HPFH) associated mutations. HbF expression and repression is a complex process governed by a multitude of genes and can function through different independent pathways [102,103]. Multiple studies have shown favourable efficiency and safety of increasing γ-globin by disrupting the BCL11A gene (Wu, Zeng et al. 2019). On the other hand, CRISPR-CAS9 was alsCRISPR-CAS9 was also used to disrupt a region of 13.6 ko composed of Δ and β-globin genes and an intergenic HBF silencer γ-simply, which led to a reactivation of HBF Summary in erythroblasts and overall improvement of the SCD phenotype [104]. Disturbance Promoting regions for HGB1 and HGB2 genes (which make the two γ-Globin subunits), significantly reduces the ability of transcriptional repressors of the link, Increase the expression of HBF [105,106]. Interesting fact, a study comparing the different CRISPR-CAS9 strategies for inducing HBF and found that genes of genes such as KLF1 And BCL11A is a much more clinically relevant approach than disturbing the transcription factor Liaison sites such as HBG1 and HBG2, even if all the methods have resulted in HBF expression levels [107].

On December 8, 2023, the FDA approved this strategy for SCD as First editing therapy of CRISPR-CAS9 genes in the world, called CASGEVY, developed by VERTEX Pharmaceuticals and Crispr therapeutics. CASGEVY is unique therapy administered to 12-year-old SCD patients with frequent vaso-occlusive crises, used in the context of a Hematopoietic stem cell transplant [108]. A 15-year multisite safety study (NCT04208529) initiated a cohort of pediatric and adult patients from previous CTX001 clinical trials in 2021 studies (NCT03745287), including transfusion-dependent β-thalassemia and severe sickle cell disease to conduct research. This study is aimed at investigating the long-term effects of Casgevy and so far there has been no DSB-related genotoxicity observed [109]. The approval of Casgevy represents a major milestone in the field of gene therapy and editing, the first of its kind. It targets both SCD and β-thalassemia and highlights the potential of CRISPR-Cas9 technology in the treatment of genetic diseases, paving the way for future developments and applications in precision medicine.

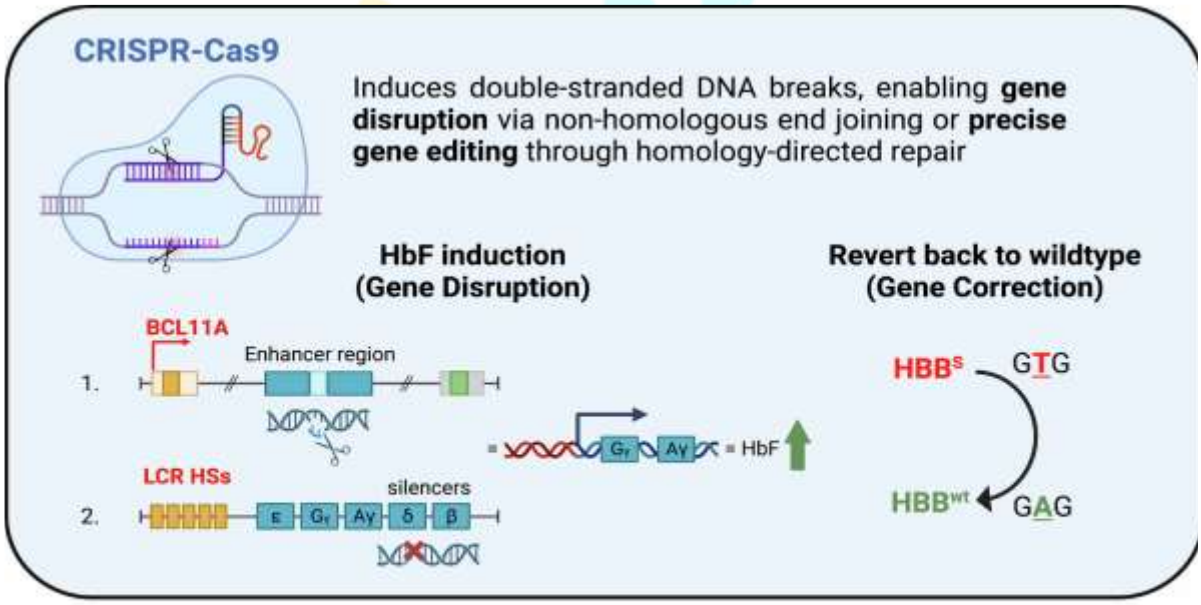

Fig 5: Gene Editing technology for SCD: CRISPR-Cas9, CRISPR-Cas9 can be used to either correct the mutation to the wild type or induce fetal haemoglobin (HbF) expression by inducing a change in the BCL11A erythroid-specific enhancer or deleting part of the HbF silencers which expression is controlled by LCR hypersensitive sites .

CRISPR-Cas9 Mechanism for Sickel Cell Anemia:

Step 1: Target Recognition

1. Guide RNA (gRNA) designed to target HBB gene mutation.
2. gRNA binds to Cas9 enzyme.
3. gRNA-Cas9 complex recognizes target DNA sequence.

The CRISPR locus is initially transcribed into a long RNA molecule known as pre-crRNA. Next, the tracrRNA hybridises with the repeat part of the pre-crRNA, resulting in the cleavage within the repeat by RNase III polymerase. As a result, mature crRNAs are formed [95].

Step 2 : DNA Cleavage

1. Cas9 enzyme cleaves target DNA at specific location.
2. Double-stranded break (DSB) is generated.

Cas9 then cleaves and forms double-strand breaks (DSBs) three base pairs upstream of the PAM sequence by using HNH and RuvC domains for cleaving the complimentary and non-complimentary strands, respectively [94,110].

Step 3: Repair Machinery Activation

1. Cells repair machinery is activated.
2. DNA repair pathways (NHEJ or HDR) are triggered.

The resultant DSBs are repaired by two mechanisms, namely non-homologous end joining (NHEJ) and HDR pathways.

Step 4: Gene Editing

1. HDR (Homologous Directed Repair): Precise editing with template.
2. Corrected HBB gene is integrated into genome.

In the NHEJ pathway, which is the dominant repair mechanism, short insertions or deletions (indels) are incorporated, resulting in frameshift or exon-skipping mutations and causing the target sequence to be disrupted. On the other hand, the HDR pathway works by utilization of a DNA donor template that is homologous to the target sequence. Due to HDR's precision in inserting or removing genes, it is widely used in CRISPR-Cas9 gene editing [112].

Step 5: Corrected Haemoglobin Production

1. Corrected HBB gene produces normal haemoglobin.
2. Red blood cells no longer sickle.

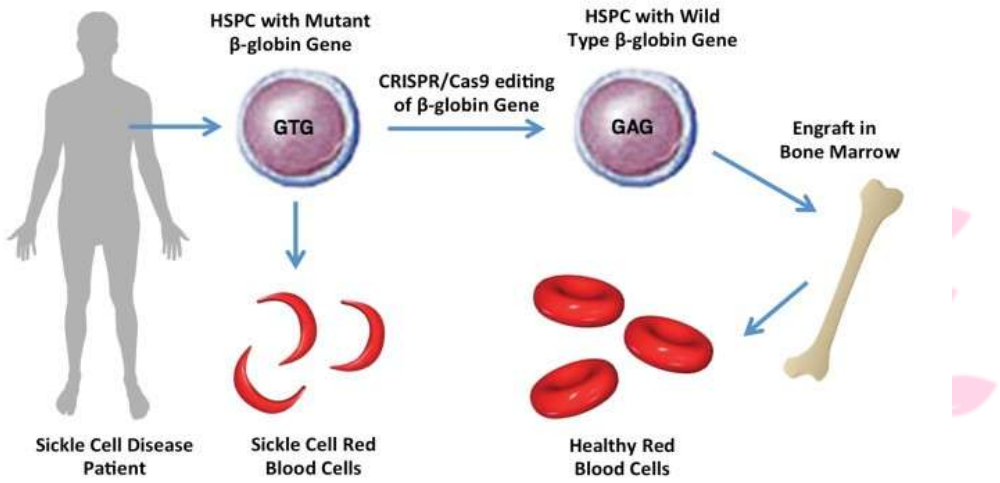

Fig 6: CRISPR-Cas9 mechanism for sickle cell anemia

CRISPR-Cas9 delivery methods for sickle cell anemia:

Viral Vector- Based Delivery

1. Lentiviral vectors (LVs): Efficiently transduce hematopoietic stem cells (HSCs).
2. Adeno-associated viral vectors (AAVs): Target HSCs and provide long-term expression.
3. Retroviral vectors: Efficiently transduce HSCs, but may have safety concerns.

Non-Viral Delivery Methods

1. Electroporation: Uses electrical pulses to deliver CRISPR-Cas9 to HSCs.
2. Nanoparticles: Employ nanoparticles to deliver CRISPR-Cas9 to HSCs.
3. Lipofection: Uses liposomes to deliver CRISPR-Cas9 to HSCs.
4. Microinjection: Directly injects CRISPR-Cas9 into HSCs.

Cell specific delivery

1. Hematopoietic stem cell (HSC) – specific promoters: Drive CRISPR-Cas9 expression in HSCs.
2. CD34+ cell-specific delivery: Targets CD34+ HSCs for efficient editing.

In Vivo Delivery

1. Hydrodynamic injection: Delivers CRISPR-Cas9 to liver or other organs.
2. Systemic delivery: Uses nanoparticles or liposomes to deliver CRISPR-Cas9 systemically.

Ex Vivo Delivery

1. Bone marrow transplantation: Edits HSCs ex vivo and transplant into patients.
2. Peripheral blood editing: Edit peripheral blood cells ex vivo and reinfuse into patients [113,114].

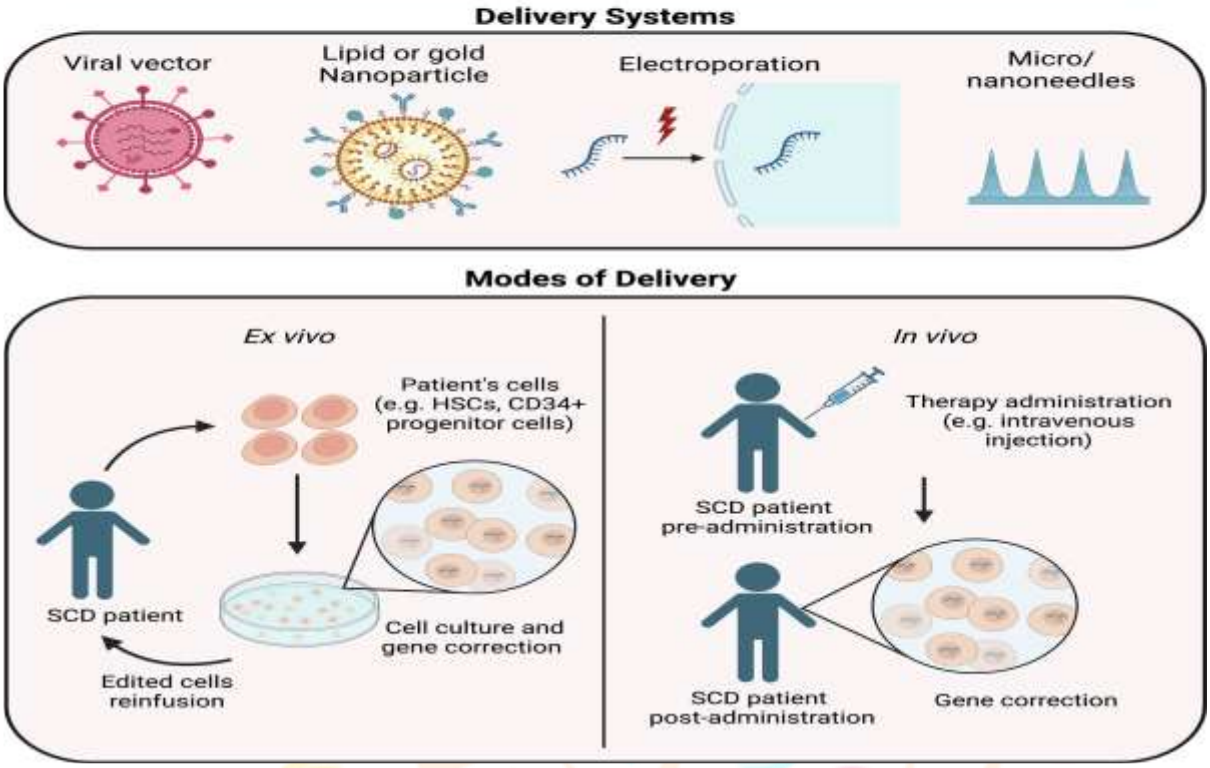

Fig 7: delivery methods- Schematic showing different aspects of gene editing delivery method that can be combined to design a gene editing strategy for sickle cell disease.

Future considerations:

In the treatment of sickle diseases (SCD), two promising strategies must be underlined: pre-born Hematopoetic therapy of stem cells (HSC) and umbilical cord blood, especially using Edition of CRISPR-CAS12 genes. Non -invasive prenatal diagnoses could allow sooner, safer Interventions with CRISPR-CAS12 offer specificity and effectiveness of gene treatment.

Nordblut -HSCS offers a unique double chance: as a source for the two Allogens Transplantation, requires an improvement in collection and preservation and autologous methods Transplantation according to the CRISPR-CAS12 treatment for correction of the mutation of the HBB gene. Such Treatments, in particular autologous transplantation, require a strict preclinical and clinical Examination for safety and efficiency.

CRISPR-Cas12's potential over other gene-editing tools like Cas9 lies in itsspecificity and efficiency [96,117], warranting research into its application in editing the HBB gene in cord blood stem cells and exploring innovative delivery mechanisms.

Addressing the implementation of these therapies in under developed regions, particularly in Africa where SCD is prevalent, highlights the need for affordable, culturally sensitive, and ethically sound approaches. This includes improving healthcare infrastructure, addressing cultural and ethical concerns, and focusing on preventive measures and early diagnosis to manage SCD effectively.

Overall, advancing SCD therapy through these avenues requires comprehensive research, ethical diligence, and collaborative interdisciplinary efforts to ensure safe, effective, and accessible treatments.

Practice points

1. Early diagnosis: Focus on early detection through non-invasive prenatal testing to enable timely intervention.

2. Informed consent: Ensure clear informed consent protocols for prenatal therapies, describing all potential risks and benefits.

3. Safety protocols: Implement strict safety measures for gene editing, with an emphasis on minimizing off-target effects.

4. Collaboration: Promote teamwork between various specialists for a comprehensive therapeutic approach.

5. Clinical trials: Conduct extensive clinical trials to evaluate the safety and efficacy of CRISPR-Cas12 before general application.

6. Regulations regulations: Follow the regulatory guidelines to guarantee an ethical and safe gene Treatment practices.

7. Follow -up: long -term follow -up for patients treated with genes to monitor treatment Results and effects.

8. Cost analysis: assess the economic feasibility of gene treatment, taking into account the two costs and possible savings.

9. Public Engagement: Speak to the public to increase awareness and acceptance of Understanding Gene Editing.

10. Funding: Look for different funding sources to support research. 11th CrisPR-CAS12 research: Develop specific guidelines for Crispr-Cas12, dealing with unique functions and application.

12. Data exchange: promoting the exchange of research results to promote progress.

Reasons to use CRISPR as an effective therapy for sickle cell disease:

With the development of CRISPR/Cas9 technology, autologous transplantation of genetically modified hematopoietic stem cells can provide a cure for most patients with sickle cell disease. Despite the many challenges associated with translating gene editing-based sickle cell disease treatment strategies into the clinic, such as: B. Given the need for high editing efficiency and low off-target effects, there are several potential solutions to these problems. A quantitative understanding of the genotypic and phenotypic consequences of CRISPR/Cas9-edited CD34+ SCD cells is essential for safe clinical applications. Developing strategies to generate long-term polyclonal hematopoietic stem cells that retain a high proportion of genetically modified cells after transplantation remains a challenge [118].

Genome editing is a revolutionary and potentially useful therapeutic method that can be used to treat patients with thalassemia. Includes the use of targeted nucleasis to repair mutations, In particular DNA sequences and return the order to its type of natural game.CRISPR/CAS9 is a powerful gene elaboration technique that is used in a variety of genetic manipulation Initiatives. It is an efficient and precise technique, which makes it suitable for the genome edit. The elaboration of the Crispr/Cas9 genome is a powerful technology capable of restoring –Globin function without causing negative effects. You can use CRISPR/CAS9 to reduce BCL11a - Spression, with consequent increase in the production of HBF. Although these genome editing technologies are being evaluated in vitro, their full potential remains unknown. CRISPR/Cas9 can be used to fine-tune transcription, edit the genome, and alter epigenetics [119].

Furthermore, the CRISPR-Cas9 system offers several important advantages, including ease of design and cloning, high accuracy of genome editing, and the ability to multiplex, allowing simultaneous targeting of multiple loci [120].

Conclusion:

SCD is a complex genetic disease that affects millions of people worldwide. This debilitating the condition is characterized by abnormally shaped red blood cells, which leads to a wide range to complications. Understanding the causes, symptoms, and treatment options for SCD is critical to improving the quality of life for those with SCD. The causes of SCD are rooted in genetic mutations that result in the production of abnormal hemoglobin.

Therapy for SCD focuses exclusively on preventing or reducing the production of HbS the amount of HbS. Available, FDA-approved treatment options include hydroxyurea and L-glutamine, with other options L-glutamine, crizanlizumab, and voxelotor as second-line therapy. In addition to the above treatment options, there is a blood transfusion treatment for certain cases. HBB deletion and BCL11A mutation using the CRISPR/Cas9 genome editing method can be a promising solution as a current therapy for SCD .Besides being faster, cheaper, and highly effective,this therapy is also believed to have great potential to provide therapy with permanent effects. It is hoped that further research will be carried out to determine the effectiveness of HBB and BCL11A removal therapy with CRISPR/Cas9. This method is clinically tested to determine the possible side effects of its use. This therapy may subsequently be used as the main therapy for patients with SCD.

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