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Gene Editing in Management of Sickle Cell Disease

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Abstract
Sickle cell disease (SCD) arises from a prevalent monogenic disorder caused by a mutation in the β-globin gene, resulting in the production of abnormal hemoglobin S (HbS) and leading to diverse complications such as chronic anemia, inflammation, vaso-occlusive crises, pain, stroke, and reduced life expectancy. Existing treatments offer temporary relief, but gene therapy, particularly utilizing CRISPR-Cas9, holds promise in addressing the underlying cause. Despite potential risks, this evolving therapeutic approach brings optimism for a more efficient and safer SCD treatment.

Keywords: *Gene Editing, Management, Sickle Cell Disease*

Introduction

Sickle cell disease is one of the most common disorders caused by a mutation in a single gene, affecting millions of individuals around the world. There are approximately 100,000 sickle cell disease patients in the United States, and one in every 365 African American babies is born with this disease (1). The disease is characterized by a malfunction in a protein called hemoglobin, the component of red blood cells that is responsible for carrying oxygen throughout the body. Individuals with this disease produce a defective version of hemoglobin called hemoglobin S, rather than the normal version (hemoglobin A) (2).
The round cells are the red blood cells with hemoglobin A, and the thin sickle cells have the mutant hemoglobin S. That mutation causes the amino acid valine to be produced instead of glutamic acid, and this causes irregularity in both the shape and function of the protein. The version of this gene containing the mutation is often referred to as the sickle hemoglobin (HbS) gene because of the hemoglobin S that it encodes. The disease is inherited autosomal recessively, the mutation must be present in both copies of the gene for the individual to have sickle cell disease (3).
The sickle shape of the red blood cells can cause them to get stuck on blood vessel walls, build up, and eventually break down prematurely. This can lead to bacterial infections and strokes. The lack of healthy red blood cells (anemia) can lead to an insufficient amount of oxygen being delivered to vital organs (2). Vaso-occlusive crises (when circulation is blocked to a certain organ) often

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occur in sickle cell disease patients because of the sickle cell buildup in the vessels. This leads to a lack of oxygen in that organ and causes a period of excruciating pain (3).

Sickle cell disease (SCD) is an inherited monogenic disorder characterized by a single substitution on chromosome 11 where glutamic acid is replaced by valine in the sixth codon of the β-globin gene. Whether inherited either in a homozygous state or with another abnormal β-globin gene, SCD encompasses a group of disorders with variable clinical phenotypes yet share a common pathophysiologic consequence derived from a single monogenic change. The modified β-globin gene produces an abnormal hemoglobin S (HbS) which rapidly polymerizes in the deoxygenated state altering red blood cell (RBC) rheology and lifespan. This single substitution leads to multiple downstream effects and devastating clinical complications including chronic anemia, chronic inflammation, recurrent vaso-occlusion, acute and chronic pain, stroke, organ failure, and early mortality (4).

Sickle cell disease causes life-long complications leading to significant morbidity and a shortened life expectancy. The classical mutation causing SCD, also known as hemoglobin SS disease, is a homozygous point mutation (A-T) in the sixth codon of the β-globin gene on chromosome 11. This mutation leads to a single amino acid substitution of glutamic acid to valine at position six within the β-globin chain. This single substitution characterizes sickle hemoglobin (HbS) and leads to the many clinical manifestations. Persons with hemoglobin SS disease do not synthesize hemoglobin A (HbA) and instead have >75% HbS. Deoxygenation of HbS leads to polymerization of the hemoglobin, followed by the formation of a gelatinous network of fibrous polymers and the transformation of red blood cells (RBCs)into rigid, sickle-shaped cells. Coinheritance of hemoglobin C, hemoglobin E, or β-thalassemia alleles (including β-thal0 or β-thal+, among others) leads to forms of SCD with varying phenotypes (5,6).

The sickled RBCs, being less deformable, cause capillary blockage, sustain cell membrane damage, and undergo hemolysis, all of which contribute to the clinical manifestations of SCD. The phenotypic presentations of patients with SCD range significantly in severity, including both acute and chronic complications. Most individuals in developed nations are diagnosed early in infancy via newborn screening. If undiagnosed at birth, SCD may begin to manifest as early as 6 months of age, coinciding with the natural decline of fetal hemoglobin (HbF) synthesis and the onset of production of adult hemoglobin containing β-globin. Persons with SCD experience life-long intravascular hemolysis and chronic anemia. The most common acute complication is veno-occlusive events (VOEs), which are episodic microvascular occlusions (7). These events may manifest as dactylitis (mainly in infants and toddlers) bone pain and abdominal pain due to microvascular occlusion of mesenteric blood vessels, and infarction of the liver, spleen, or lymph nodes. Other acute complications include acute chest syndrome, stroke, priapism, splenic sequestration, and transient pure RBC aplasia (8).

Treatments for this disease are limited(9). Blood transfusions are performed to alleviate some of the symptoms temporarily, and therapies to prevent the side effects of sickle cell disease such as penicillin prophylaxis, pneumococcal vaccination, and hydroxyurea therapy have been performed.

However, these do not cure the disorder. Individuals who do receive such transplants are at risk for complications such as graft rejection and graft versus host disease (10).

One potentially new treatment for sickle cell disease that seems promising is gene therapy. Gene therapy is the process of introducing genetic material into cells with the intention of causing some sort of therapeutic effect. These effects may include fixing a mutated gene, inserting missing genetic information, or providing the genetic instructions for that cell to perform a different function. One of the most common methods of gene therapy is inserting a viral vector: a virus whose DNA has been edited to incorporate a specific gene, into the cell to transfer that gene into the cell's genome (11). Another method of gene therapy involves inserting synthetic oligonucleotides into the target cells to inactivate certain genes. Synthetic oligonucleotides are short strands of DNA or RNA that are engineered to be complementary to the DNA of the particular gene to be turned off. When inserted into the cell, they bind to the DNA of that gene and prevent it from being transcribed into mRNA, ultimately preventing it from producing a protein, and thus silencing its effects. A third method of gene therapy involves inserting mRNA into the cell to be translated by ribosomes and produce a speciyc protein (12). Each of these methods comes with serious risks. For example, inserting viral DNA containing a certain gene into the cell's genome can lead to negative side effects, including cancer. Another negative side effect is immunogenic toxicity. Since the viral vector is foreign to the body,it sometimes can generate an immune response including antibodies to fight the virus. This could ultimately prevent the wanted gene from being inserted into the cell's genome, and could also lead to adverse effects, such as an allergic reaction. Both side effects could end up causing more problems than the gene therapy was initially aiming to (13).

Recently, a radical new form of gene therapy without such side effects has been developed. This form is cheaper and easier to use than others, and it incorporates CRISPR-Cas9. CRISPR stands for "clusteredregularly interspaced short palindromic repeats," and Cas9 stands for "CRISPR associated protein 9." The gene therapy developed is a modiycation of the naturally occurring system found in bacteria called CRISPRCas. It was first discovered in a type of bacteria called Escherichia coli in 1987, but its purpose was not discovered until two decades later (11).

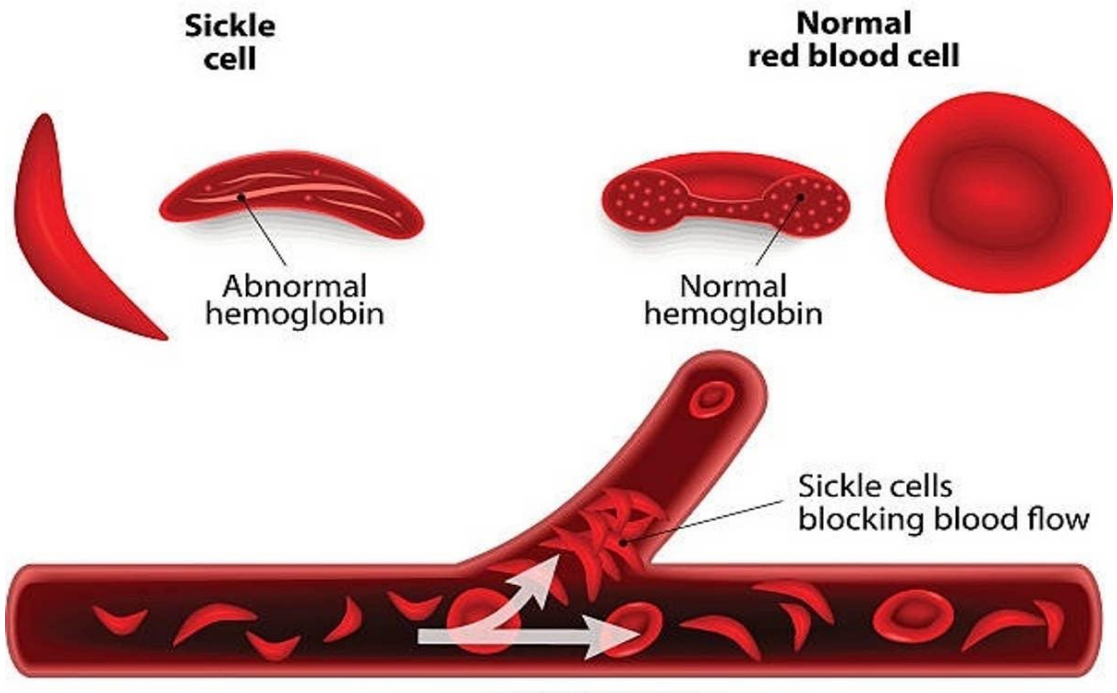

Fig. 1: Normal and sickle RBC (7).

Pathophysiology of SCD

Hemoglobin, which is made up of four globin chains packed around individual hemoglobin molecules, is a significant protein of RBCs. Hemoglobin carries O2 from the lungs to tissues,also carries CO2 from the tissues to lungs. Approximately 97% of adult hemoglobin is HbA, which has 2α and 2β globin chains. Other haemoglobins are HbA2 and HbF. The HbF% quickly declines after 12 weeks of birth and HbA and HbA2 remain as the hemoglobin. The beta globin gene is found on chromosome 11p15.5 (14). A single replacement of hydrophobic amino acid valine for glutamic acid on chromosome 11 results in SCD,a hereditary single gene illness that damages the β-globin gene's 6^{th} codon (10). This β-globin gene is found on the arm of chromosome 11. When two mutant betaglobin subunits combine, hemoglobin S is produced. Red blood cells enlarge and become less flexible because there isn't a hydrophilic amino acid which is on sixth location of the hemoglobin sequence, which promotes non-covalent clumping of hemoglobin in very less oxygen environments (15). RBCs with HbS or HbS in combination with other faulty genes go through polymerization and harden when introduced to a oxygen less environment. Stiff RBCs can have an adverse effect on endothelial vessel wall integrity and blood flow because of their enhanced density and tendency for destruction of blood cells. Stiff RBCs cause hemolysis, vaso-occlusion. It also causes infarction and tissue ischaemia. Sickle RBCs have a half-life of just 10 to 20 days only. Healthy hemoglobin adapts to the carbon dioxide molecules it interacts with during deoxygenation, returning to its original shape upon release. HbS, on the other hand, has a tendency to polymerize into tactoids, these are gel-like substances. Whereas Hb are crystals, and hard,

insoluble threads (16). The main feature of the pathophysiology of this disease is the loss of RBSc flexibility. Normal RBCs are quite elastic; they can bend and flow via capillaries. In sickle cell disease, low levels of oxygen in blood encourages the sickling of RBCs, and frequent sickling weaken and destroy the red cell. Thus, when the oxygen pressure returns to normal, these cells do not reform (15).

The complex pathophysiology of Sickle Cell Disease stems from polymerization of Hemoglobin S in times of hypoxemia, dehydration, acidosis, and pyrexia. Polymers of HgbS cause the sickle appearance of RBCs and result in hemolysis, abnormal rheology, cellular adhesion, and decreased availability of nitric oxide. These developments result in anemia, vaso-occulsion, and vasoconstriction which are causes of sickle cell related end organ damage (16).

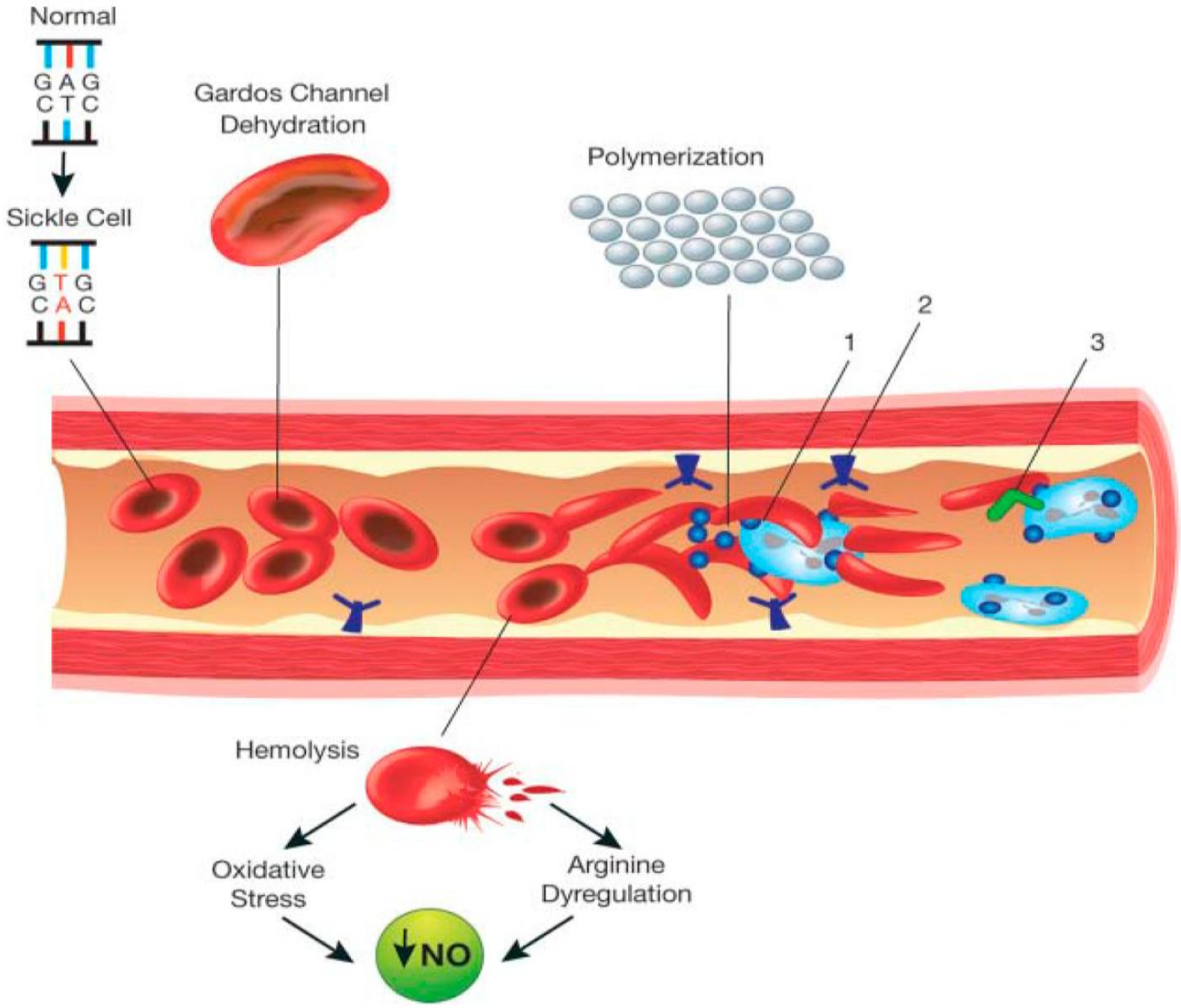

Fig 2: Schematic representation of the pathophysiology of sickle cell anemia. A single gene mutation (GAG→GTG and CTC→CAC) results in a defective haemoglobin that when exposed to de-oxygenation polymerizes resulting in the formation of sickle cells. Vaso-occlusion can then occur (16).

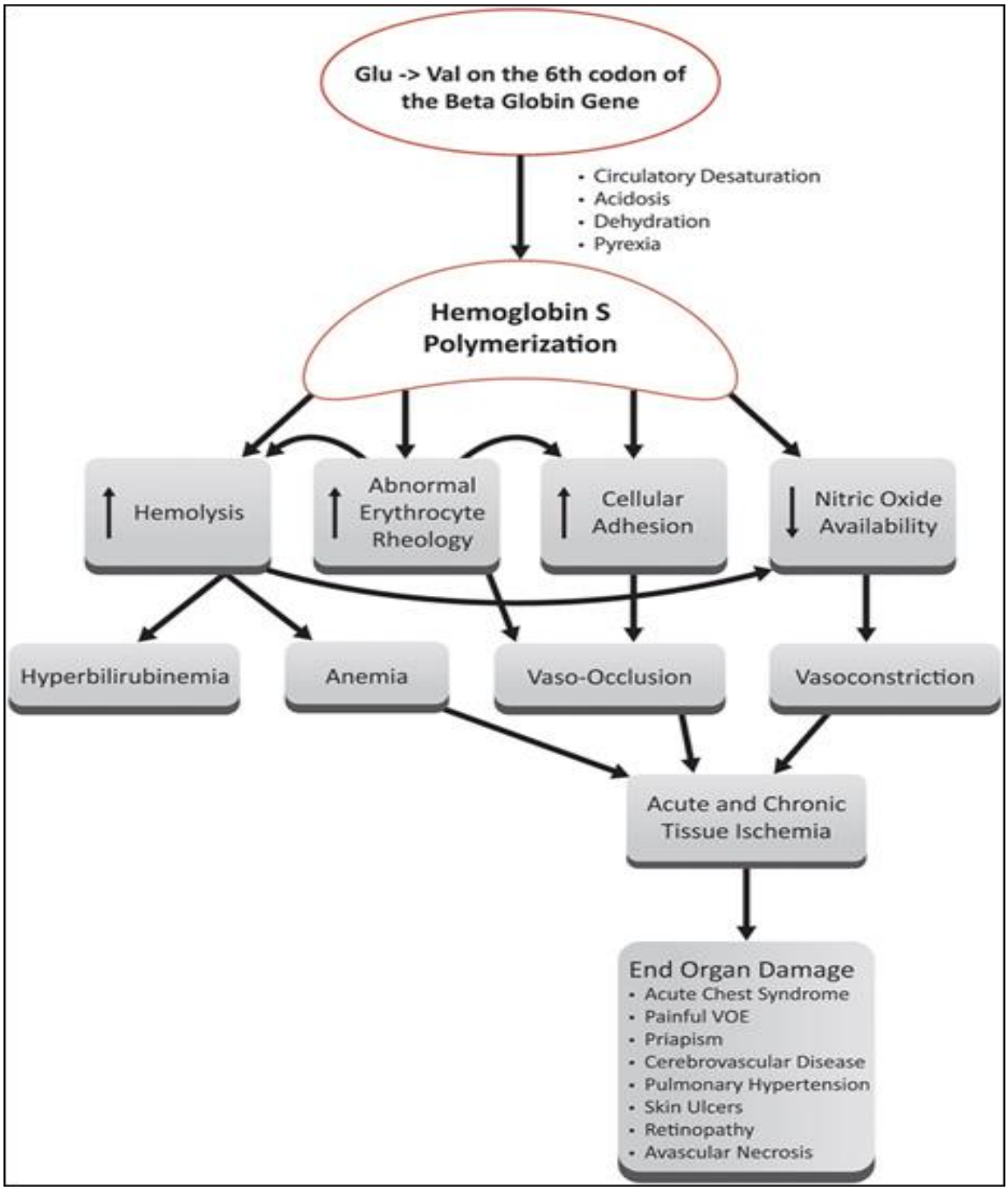

Fig 3: Pathophysiology of Sickle Cell Disease (16).

Gene Therapy used in the Management of SCD

One potentially new treatment for sickle cell disease that seems promising is gene therapy. Gene therapy is the process of introducing genetic material into cells with the intention of causing some sort of therapeutic effect. These effects may include fixing a mutated gene, inserting missing

genetic information, or providing the genetic instructions for that cell to perform a different function. One of the most common methods of gene therapy is inserting a viral vector. A virus who's DNA has been edited to incorporate a specific gene, into the cell to transfer that gene into the cell's genome (11).

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Types of Gene Therapy

Two general approaches to gene therapy are currently applied to SCD which are:

1. Gene addition
2. Gene editing (17).

Gene Addition:

Gene addition is a form of gene therapy technique being explored for single-gene diseases/disorders where a mutation occurs in one or both sets of a genes. This gene therapy technique usually involves the insertion of functional (or healthy) copies of a gene (known as a transgene) into a person's cells by way of a vector (17).

Vectors deliver the functional gene to the patient's cells, either in vivo or ex-vivo. Once inside the cell, the transgene provides the cell with instructions that lead to the production of functional proteins. With gene addition therapy, the mutated gene does not need to be replaced or removed. This provides the cell with the instructions that lead to the production of functional genes, while not needing to replace or remove the mutated gene (18,19).

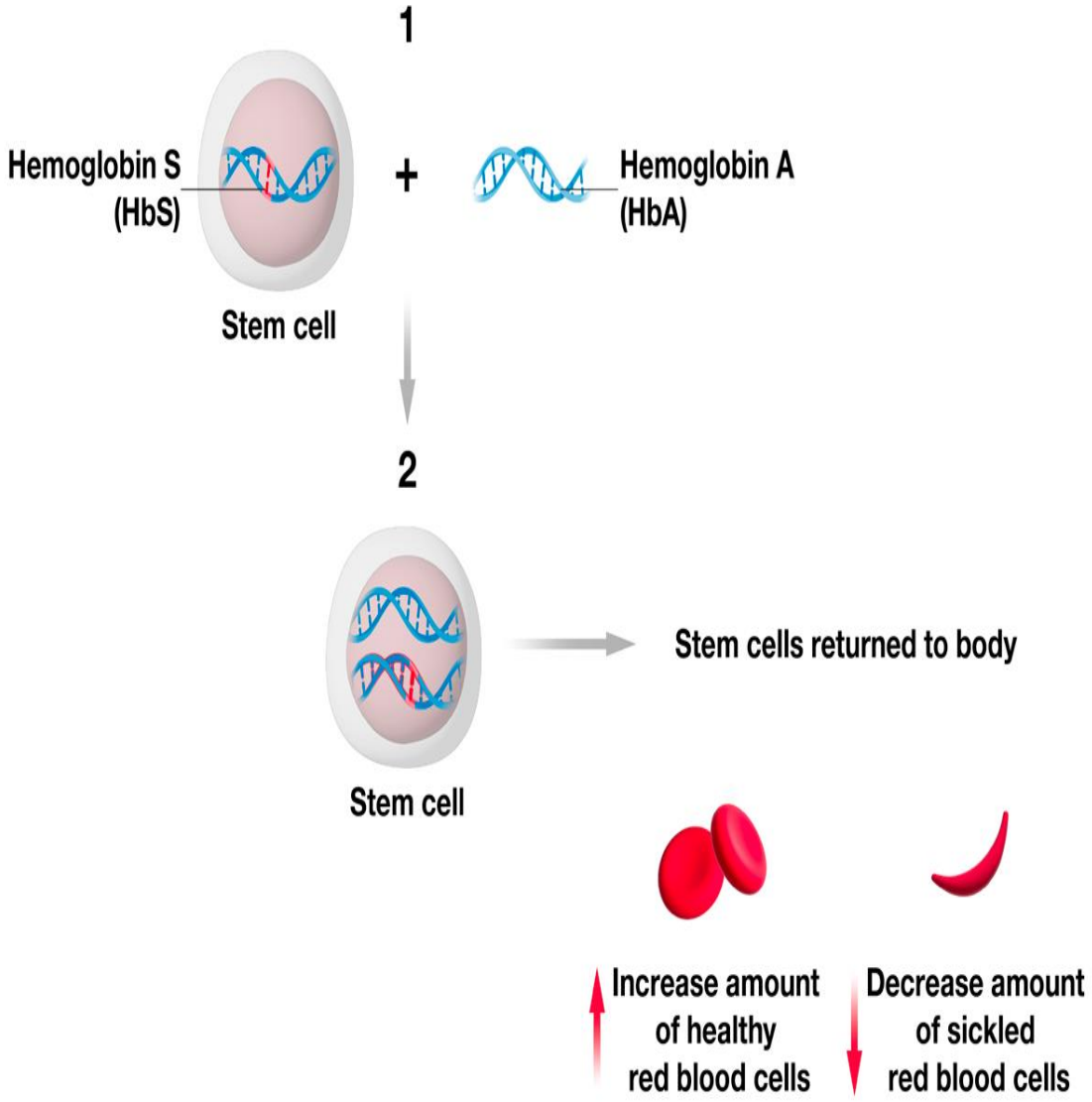

Fig.4: Patient stem cells are collected and taken to a lab for modification. An extra copy of hemoglobin A gene (without the variant) is added to the stem cell, which allows the cells to produce hemoglobin A (non-sickling hemoglobin) (18,19).

Gene Editing in Management of SCD

Genome editing is desirable for management of SCD as it leads to permanent removal or correction of a detrimental mutation, or by the creation of protective insertions or deletions. Theoretically, programmable nucleases create double strand breaks (DSB) at a specific genomic locus followed by recruitment of DNA repair mechanism through either non homologous end-joining (NHEJ) or homology directed repair (HDR) (using homologous sequences found in sister chromatids, homologous chromosomes or extra chromosomal donor DNA sequence provided for correction purposes) to the DSB site (20).

Gene editing involves the creation of targeted breaks in the DNA, with or without instructions to repair them, through a number of different techniques,it uses uses a small segment of RNA, termed "guide RNA," to find a specific DNA sequence within the human genome, using the CRISPR molecule as a platform. An attached enzyme (Cas9) then cuts the DNA at a precise location or alters specific nucleotides (18,19)

Types of Gene Editing

There are 2 primary techniques in gene editing

1. Gene silencing/ disruption/inactivation
2. Gene correction /insertion.

While each of these approaches introduces different types of gene-based changes, they start out the same: stem cells are collected and taken to a lab for modification. After modifications are made in a lab, stem cells are returned to the body. These approaches aim to decrease the amount of hemoglobin S in red blood cells. If they successfully decrease the hemoglobin S,the cells will not be fragile or even sickle. As a result, these approaches can decrease the complications, treat or prevent symptoms of sickle cell disease. However, this type of gene therapy will neither reverse some complications (such as avascular necrosis) if they are present at the time of the gene therapy nor alter the genes that is pass on to the children(10).

1. Gene silencing/ disruption/inactivation:

Gene disruption or gene inactivation are sometimes referred to as gene silencing, knockdown, or knockout. The technique occur by disrupting or inactivating genetic material that is responsible for the genetic disease. This can be achieved by turning off genes that cause disease or disrupting a separate gene that will compensate for the disease-causing gene (20).

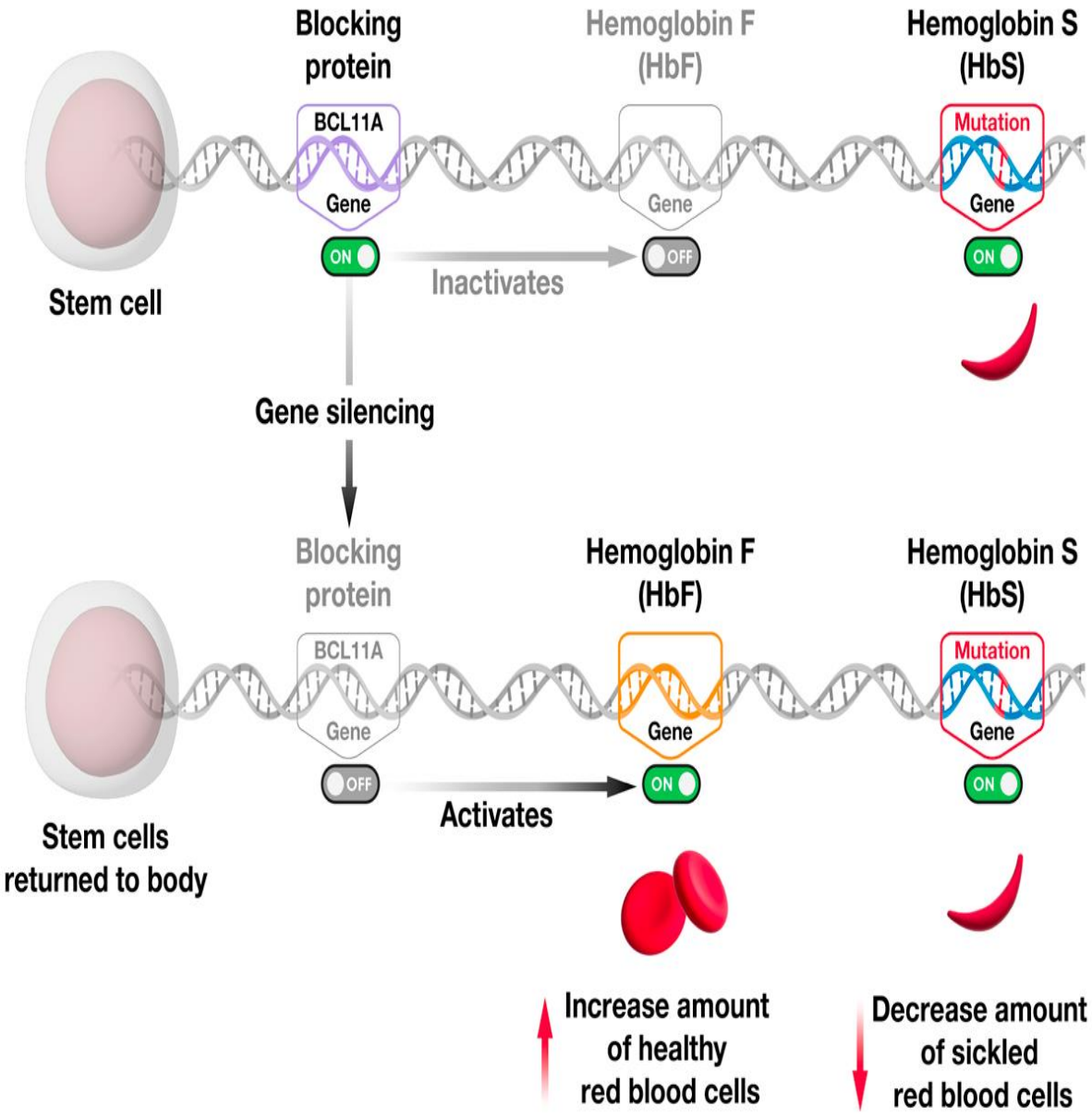

Fig. 5: Patient stem cells are collected and taken to a lab for modification. The gene that produces the BCL11A-blocking protein, which inactivates hemoglobin F, is silenced. By silencing this gene, the gene that makes hemoglobin F can be activated, which allows cells to produce hemoglobin F (non-sickling) (20).

Gene correction

In this technique genetic material are corrected by creating a break in the gene and providing a corrective template or inserting new genetic material for the cell to use to repair the mutated gene (21).

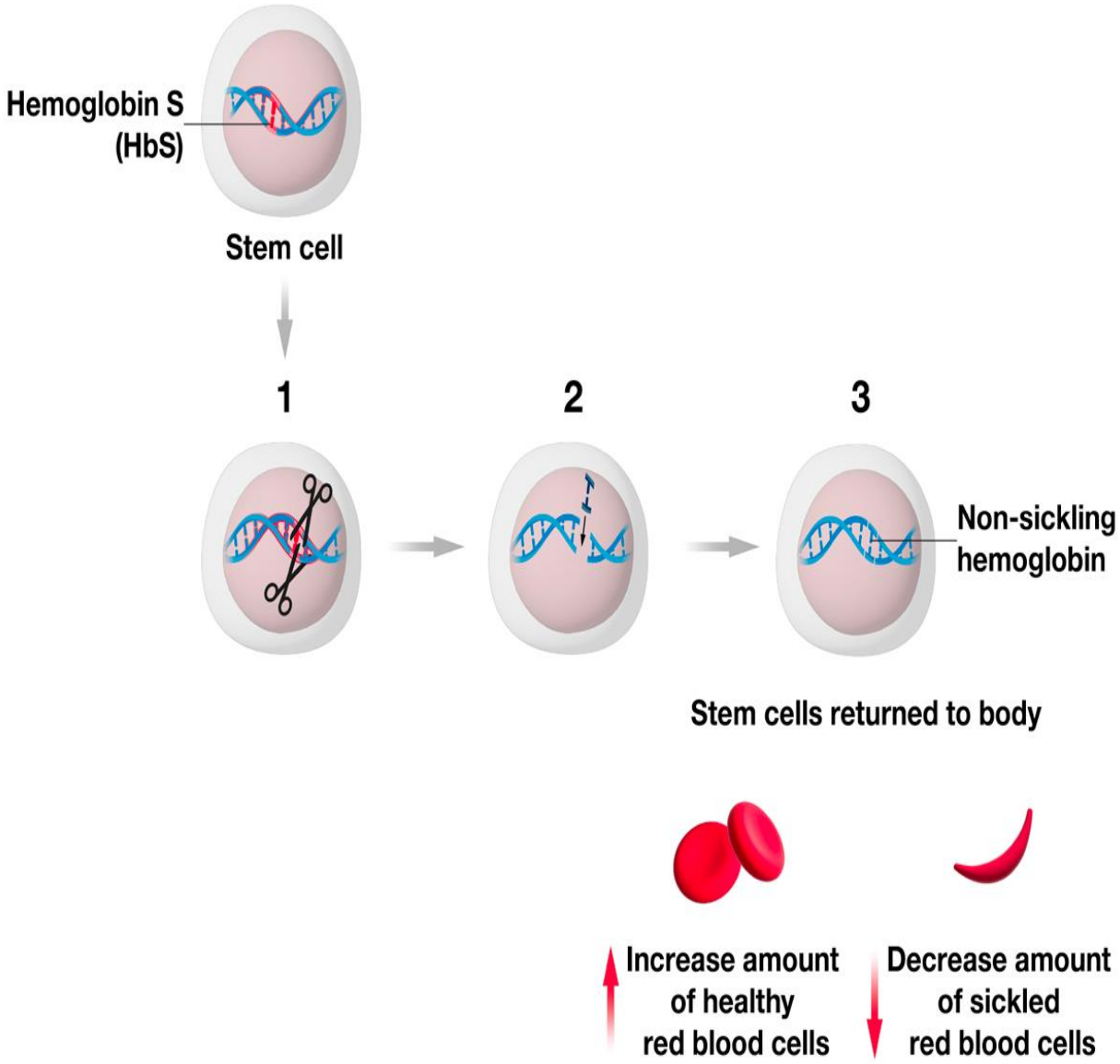

Fig. 6: Patient stem cells are collected and taken to a lab for modification. The variant in the gene that causes sickle cell disease is corrected so that it codes for a non-sickling hemoglobin (21)

Major nucleases used for gene editing technique

1. Meganucleases also known as homing endonucleases
2. Zinc finger nucleases (ZFNs)
3. TAL-effector nucleases (TALENs)

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4. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) (22)

These tools have been successfully used ex vivo to correct the SCD mutation and induce fetal globin by editing regulatory sequences such as promoters or other regulatory sequences including BCL11A, KLF1 and MYB to circumvent the severity of the mutation in sickle HSPCs (22).

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9), uses a specific RNA (guide RNA) sequence recognizes the target DNA region of interest and directs the effector Cas protein there for editing. It was discovered that CRISPR/Cas is an acquired defense mechanism in opposition to pathogens in archaebacteria via DNA recognition by CRISPR RNA (crRNA) and enzymatic DNA cleavage by Cas nucleases. Prokaryotes, which include bacteria and archaea, have a wide range of defense systems to fend off intruders from outside their own species, particularly viruses. Once introduced into target cells, CRISPR/Cas9 directed DSBs result in activation of DNA repair mechanisms. This machinery would lead to either some insertions/deletions (INDELs), which ideally results in loss-of-function for a given gene, or would repair the DNA break using homology strands if HDR is activated. In this manner, CRISPR/Cas9 technology can target correction of the SCD mutation or induce fetal hemoglobin expression by editing chromosomal areas controlling its expression (23).

Mechanism of Gene Editing

During gene editing, a site-specific double strand breaks (DSB) is usually created with targeted nucleases, such as zinc finger nucleases, transcription-activator-like effector nuclease (TALENS), meganucleases, and Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system (24).

The DSB can be repaired by any one of the following methods: (i) non-homologous end joining (NHEJ) with direct fusion of the nuclease cleaved ends. This method is quite error-prone resulting in genomic insertions and deletions, and (ii) homologous directed repair (HDR), using an exogenous template to insert, delete, or replace a genomic sequence accurately (24). Use of these site-specific nucleases actually facilitated effective gene targeting to amend sickle mutation in human induced pluripotent stem cells (iPSCs) (25).

Genome modification typically ensues from DNA double strand breaks (DSBs) that are introduced by programmable designer nucleases, such as zinc finger nucleases (ZFNs) (26), transcription activator-like effector (TALE) nucleases (TALENs) (27,28), or the CRISPR-Cas system (29). Other than the entirely protein-based ZFNs and TALENs, CRISPR-Cas nucleases contain an engineered guide (gRNA) that is complementary to the desired target sequence and that directs the Cas protein to the chosen genomic locus to induce a DSB (30).

Non-homologous end joining (NHEJ) and HDR are the two major repair pathways triggered by DSB formation. NHEJ is a fast but error-prone pathway, leading to insertions and deletions at the break site. NHEJ is hence typically employed to disrupt genes or *cis*-regulatory elements with high efficacy, reaching editing frequencies of over 90% in HSCs (31).

In contrast, Homologous Directed Repair is a slow but precise DNA repair pathway that uses a co-introduced DNA fragment as a template to correct disease underlying mutations. In HSCs, the

HDR template is typically delivered by vectors based on adeno-associated virus (AAV) (32) or in the form of single-stranded or double-stranded oligonucleotides (33). However, because HDR is restricted to the S/G2 phase of the cell cycle, achieving gene targeting frequencies that exceed 20% in mainly quiescent long-term repopulating HSCs remains challenging (34). Due to the genotoxic potential arising from DSB formation, alternative platforms to edit the genome have been sought for. Such strategies are mostly based on CRISPR-Cas nickases that cleave only one DNA strand (35,36). This family includes base editors (BEs) (37,38).

A Cas9 nuclease is converted to a Cas9 nickase by introducing mutations in one of the two catalytic domains of Cas9 (29). BEs were developed by fusing a deaminase domain to a Cas nickase (37).

There are two types of BEs: cytosine base editors (CBEs) convert a C-G base pair (bp) into a T-A while adenine base editors (ABEs) convert an A-T to a G-C bp. BEs can be employed to correct point mutations, to introduce stop codons, or to disrupt *cis*-regulatory elements. PEs consist of a Cas9 nickase coupled to an engineered reverse transcriptase, which transcribes a section of the pegRNA (prime editing gRNA) into DNA to introduce the desired changes, such as base conversions or insertions or deletions of up to 80 bp (39).

In CRISPR/Cas9, an RNA (guide RNA) sequence identifies the effector Cas protein, which is then aimed to the targeted DNA region of importance. This technology changed genome editing procedures and increased the likelihood of applying genome editing techniques in a clinical context because it is simple to use, efficient, and reasonably priced. Once transported into target cells, CRISPR/Cas9 mediated DSBs activate DNA repair mechanisms (22). If HDR is activated, this mechanism either results in INDELs, which results in the distruction of a gene's function, or it uses homology strands to repair the DNA break. CRISPR/Cas9 technology can be used to specifically fix the mutation of SCD by editing the chromosomal areas that regulate fetal hemoglobin expression (39)

DSBs are the foundation of traditional CRISPR-Cas9 editing, which is then followed by cellular repairs of chromosomal breaks via either NHEJ or HDR. While damaged DNA ends in NHEJ are tied up with no consideration for homology, a donor template is provided in HDR, making it a precise repair mechanism that can introduce or repair necessary mutations. Induce HbF expression was focused at very beginning for clinical trials of gene editing in SCD than direct sickle mutation correction as expressed HbF does not require HDR and in HSCs it is tough to achieve HDR (39) .

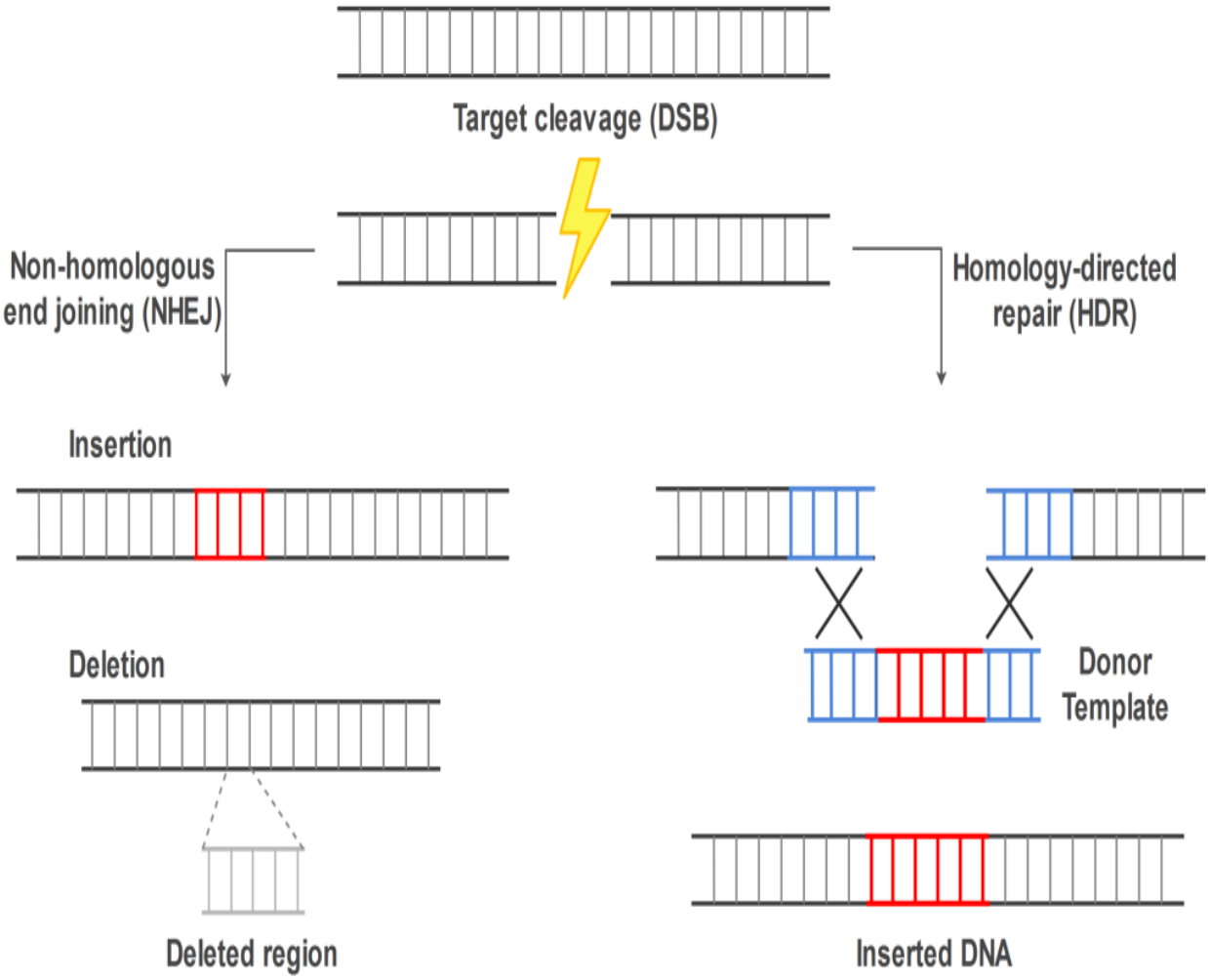

Fig. 7: CRISPR/Cas9-mediated DSB repair mechanism. The Cas9 introduces a DSB in the target DNA. DNA repairing includes the NHEJ and HDR pathways. The NHEJ pathway leads to Indel. The HDR pathway uses homologous donor DNA sequences to create precise insertion between DSB sites (40).

Nonhomologous end joining: The predominant and error-prone version of double-stranded DNA repair is nonhomologous end joining (NHEJ). With this approach, repair occurs mostly at the cost of loss of gene function in the region where the breaks happen. Double-strand breaks combined with the NHEJ that disrupts gene function can be used for re induction of HbF by removing the silencing that occurs developmentally in the transition to HbA(41)

Homology directed repair: The less common mechanism of repair, called homology-directed repair (HDR), makes use of a template to potentially restore the damaged gene's ability to encode

a normal protein. Gene-editing approaches that involve HDR require delivery of nucleic acid sequences that can be used as repair templates that introduce a desired DNA sequence where the double-strand break has been made. In this way, a genomic region harboring a mutation can be repaired by replacement with a normal one (42).

Gene Editing Approaches for Management of SCD

BCL11A gene disruption

BCL11A plays a variety of functions in distinct hematopoietic pathways, and gene disparity in BCL11A is extremely detrimental. By focusing on the centre of the BCL11A enhancer in HSPCs, several researchers have identified BCL11A erythroid booster as a candidate for inducing HbF and providing a basis for more specific to erythroid therapeutic genome editing. BCL11A expression and HbF suppression depend on a GATA1 motif that functions as the heart of an enhancer. While protecting BCL11A expression and function in non erythroid situations, specific gRNAs that disrupt the enhancer significantly increase HbF induction in primary erythroid precursors (43).

The same HbF reactivation were observed with BCL11A enhancer disruption compared to BCL11A coding deletion, but BCL11A was still able to promote HSC function, including maturation, reconstruction, and long-term engraftment prospects. By disrupting the GATA1 binding site at the +58 BCL11A erythroid promoter using CRISPR/Cas9 technology, highly effective corrective gene editing can be achieved in HSPCs. Thus,This led to therapeutic activation of fetal γ-globin in engrafting SCD HSCs and erythroid-specific decrease of BCL11A expression occurred.The highest concentrations of HbF induction were seen in erythroids with a high rate of indels when gRNA effectively cleaved the +58 erythroid enhancer of BCL11A. Biallelic alterations at the cleavage location led to a substantial induction of γ-globin.To investigate the effects of BCL11A enhancer editing on lengthy engrafting HSCs, edited human SCD CD34+ HSPCs were implanted into immune compromised NBSGW mouse. NBSGW facilitated gene editing of renewing HSCs with comparable levels of human myeloid, and erythroid engraftment of already corrected cells compared to cells that are not edited. It is clear from the persistence of indels at the BCL11A enhancer following secondary transplant that altering the BCL11A enhancer has no negative effects on stem cell function. The ability of BCL11A enhancer modified cells to sustain proposes that NHEJ-mediated gene disruption may be more effective than HDR- or Microhomology-mediated end joining-based gene editing techniques (MMEJ). This is because NHEJ primarily occurs in HSCs and is active throughout the cell cycle. This research proved that CRISPR/Cas9 editing of the BCL11A enhancer is an effective therapeutic method for producing a long-lasting therapeutic amount of HbF induction in engrafting HSCs (41).

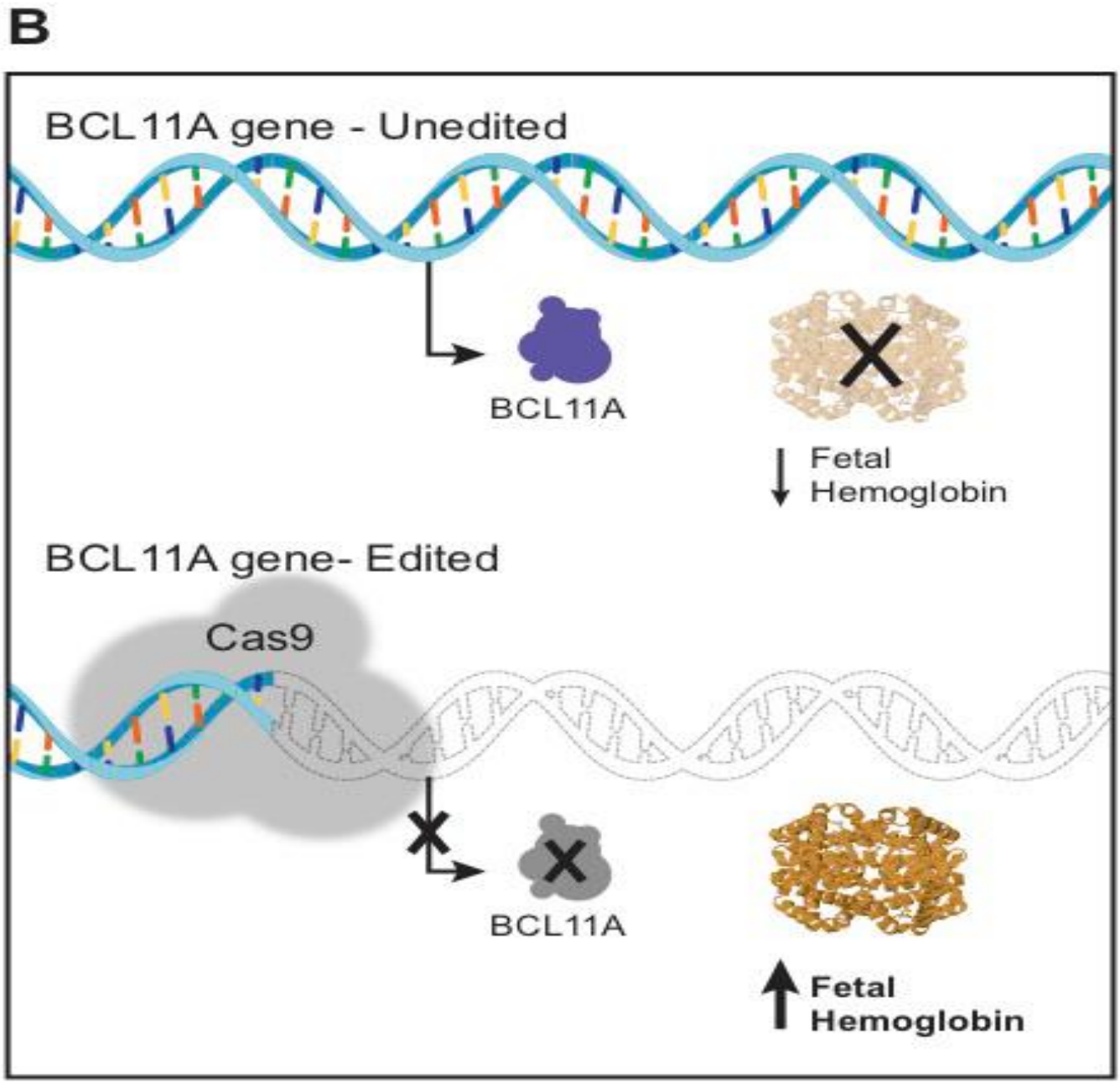

Fig.8: Schematic of gene editing to control silencing the BCL11A gene, a region of the DNA known to suppress the production of fetal hemoglobin. The use of CRISPR-Cas9 to disrupt BCL11A allows for the production of high levels of fetal hemoglobin (HbF; hemoglobin F) in red blood cells. (41).

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Direct Correction of the E6V Amino Acid Substitution in HBBS

Several groups have developed HDR-mediated approaches to correct the canonical E6V glutamic acid to valine amino acid change in β-globin that causes SCD. Using ZFN or CRISPR to introduce a DSB near the sickle-causing mutation and providing a homologous repair template that contains the wild-type base at the mutation site can revert the mutation of HBBS to HBBA, if the donor is used for HDR. Different methods have been used to provide the homologous donor, such as single-stranded oligodeoxynucleotides, or viral vectors that do not persist, such as adeno-associated virus 6 (AAV6), integrase-defective LVs, adenovirus, and others. Because of the restriction of HDR to the S and G2 phases of the cell cycle, with non-homologous end joining occurring at all times, it is challenging to achieve high levels of HDR-mediated gene correction in long-term primitiveHSCs(44).

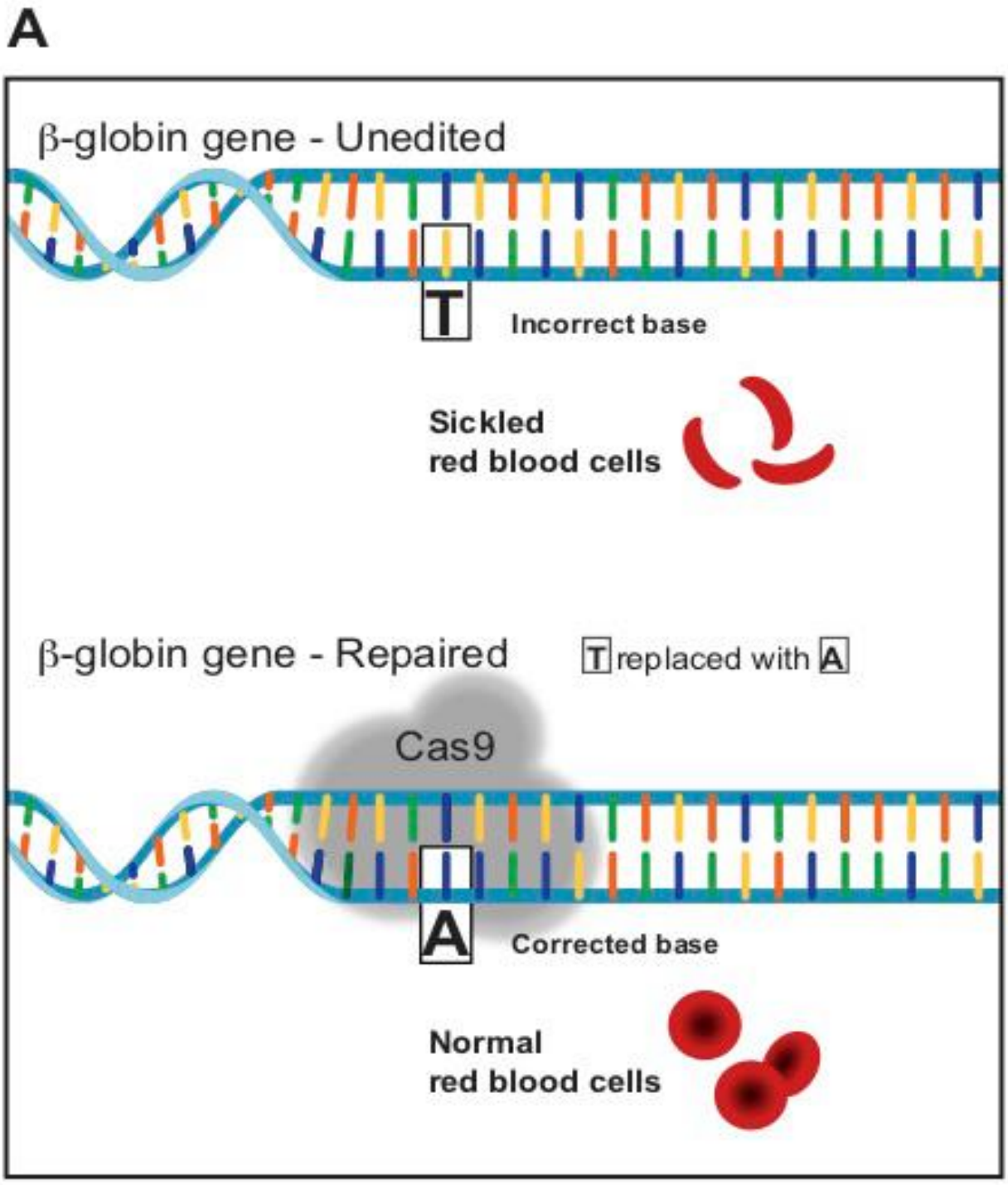
A
β-globin gene - Unedited
T
Incorrect base
Sickled
red blood cells
β-globin gene - Repaired
T replaced with A
Cas9
A
Corrected base
Normal
red blood cells

Fig.9: Schematic of β-globin mutation correction using CRISPR-Cas9. The correction is made by changing the single nucleotide mutation thymine to adenine (T to A), which changes glutamicacid to valine at codon 6 of the β-globin (*HBB*) gene (44)

Restore adult hemoglobin (HBB gene Repair)

One of the main approaches to CRISPR sickle cell gene therapy is to repair the mutation in the adult hemoglobin gene responsible for sickle cell disease, causing the healthy, normal form of adult hemoglobin (hemoglobin S) to be produced The site of the break can then be used to introduce a correction to the gene via homology-directed repair (HDR). This is called a gene knock-in, a donor template containing the normal sequence of the gene is introduced so that, the mutation is corrected when the cell repairs the DNA break with the template via HDR. The edited cells, now engineered to produce normal hemoglobin, are re-implanted in the patient's bloodstream (45).

The CRISPR-Cas9 recognizes and cuts the locus of *HBB* $^{S/S}$ in HSPCs and DSBs are induced, triggering DNA repairing. At the presence of DNA donor as a template, DSBs are repaired by HDR and the *HBB* $^{S/S}$ is converted to *HBB* $^{S/A}$ or *HBB* $^{A/A}$ (45).

HbF Induction

After birth, BCL11A binds to the γ-globin promoter directly, repressing γ-globin expression, a component of HbF that has anti-sickling effects. *HBB* $^{S/S}$ in patients produces sickle β-globin. CRISPR-Cas9 or ZFNs targeting *BCL11A* ESE, will induce DSBs and NHEJ. As a consequence, *BCL11A* expression and the inhibition to γ-globin is disrupted, leading to an increase in γ-globin. SCD, sickle cell disease; HbF, fetal hemoglobin; ESE, erythroid-specific enhancers; HbS, sickle hemoglobin (45).

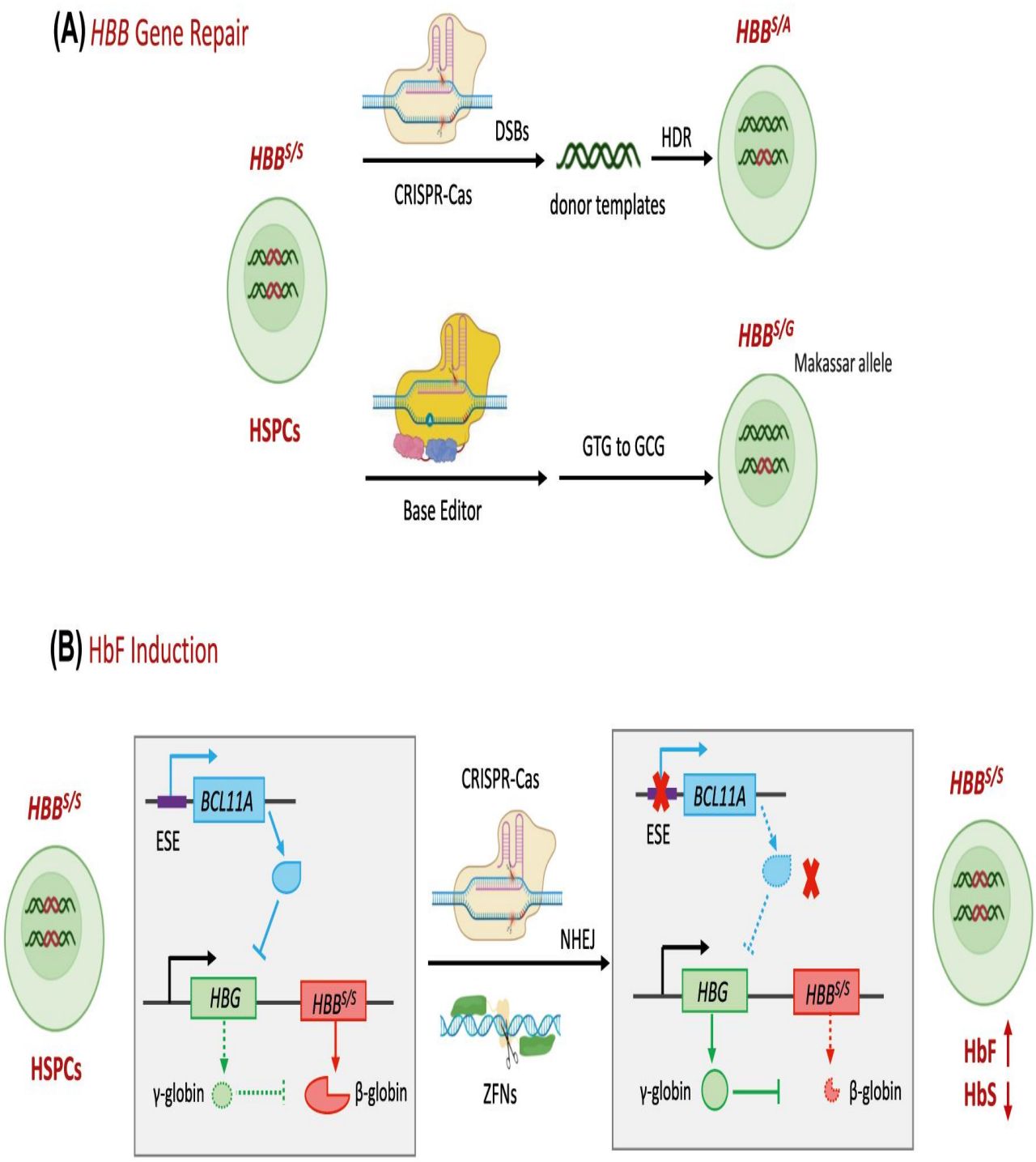

Fig.9: (A) *HBB* gene repair (B) HB F Induction (45).

Challenges of Gene Editing Technology

1. Gene delivery and activation: Delivering a gene to the wrong tissue would be inefficient, and it could cause health problems for the patient.For example, improper targeting could incorporate the therapeutic gene into a patient's germline, or reproductive cells, which ultimately produce sperm and eggs (46).

2. Disrupting important genes in target cell: Ideally, an introduced gene will continue working for the rest of the patient's life. For this to happen, the introduced gene must become a permanent part of the target cell's genome, usually by integrating, or "stitching" itself, into the cell's own DNA. But problem arises if the gene stitches itself into an inappropriate location, disrupting another gene (46)

3. Expensive: Gene therapy can be extremely pricey, making it inaccessible for some people. A CRISPR therapy for Sickle cell anemia (SCA) is expected to receive Food and Drug Administration approval in 2023 is speculated to potentially exceed Hemgenix's pricing because of the $4 to $6 million cost of lifetime treatment for severe SCA. These extraordinary costs place gene therapies primarily within the reach of society's most advantaged while excluding much of the population (46).

Conclusion

Gene editing is a process where sections of target DNA are modified by point mutation such as insertion, deletion, modification, or replacement. There are 2 primary techniques in gene editing which are gene silencing or disruption and Gene correction or insertion. Gene editing involves the creation of targeted breaks in the DNA, with or without instructions to repair them, through number of different techniques. CRISPR/Cas9 or zinc finger nucleases (ZFNs) have proven to be useful tools to delete or replace sequences in the production of hemoglobin. The repair of DNA double-strand breaks occurs through non-homologous end joining or homology directed repair which is facilitated by providing the cells with therapeutic donor DNA.

Gene editing leads to a cure for sickle cell disease, as it leads to permanent removal or correction of mutation, or by the creation of protective insertions or deletions.

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