

Research Article

# CRISPR-Cas9 Gene Editing Therapy, a Curative Hope for Sickle Cell in Nigeria, West Africa

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## Abstract

Sickle cell anaemia is one of the haemoglobin abnormalities resulting from a genetic mutation—it is caused by inheriting two faulty genes that result in an abnormal substitution of glutamate for valine on the beta chain of haemoglobin, which causes haemoglobin molecules to stick together. According to a World Health Organization (WHO) report, 20 out of every 1,000 births suffer from sickle-cell anaemia, and 24% of Nigerians are carriers of this mutant gene. Scientists have suggested several solutions, including stem cell transplantation and gene therapies, but these have faced opposition due to ethical beliefs, high cost, and the ensuing immune issues. Research is now centered on advancing genome editing techniques for gene therapy. Ongoing studies have proven that genetic differences can be corrected methodically by modifying the genome at specific sites instead of introducing a new copy of the affected gene into the cells; due to the effectiveness of this method, scientists are testing its applications in manipulating genes in various systems. This review correlates a few studies that used the recently developed technique—CRISPR-Cas9—as a novel approach to gene therapy, dissecting the different clinical studies about sickle cell origin to point out many of its ethical and medical limitations, the consequences of these limitations, and the advancements this technology has made possible.

## Keywords

Sickle-Cell Anaemia, CRISPR-Cas9, Genome Editing, Gene Therapy, Cas-9 Enzymes

## 1. Introduction

Gene therapy is a scientific method to treat diseases caused by underlying genetic abnormalities. This procedure involves altering genetic code to replace or inactivate disease-causing genes [1]. However, due to the limited success rate recorded by this procedure, it is opinionated only as a last resort and still a part of clinical trials [2]. Recently, scientists have ensured a breakthrough in science by examining the biological system through the lens of a microbe, *Escherichia coli*, dis-

covering a CRISPR system called Cascade [3]. This system is a high-precision technique that proffers adaptive immunity in prokaryotes against viruses and extrachromosomal DNA [4]. This technology was advanced by discovering the Cas9 enzyme in the human pathogen *Streptococcus pyogenes* [5]. Together, the two dynamic natures of CRISPR-Cas9 have led scientists to explore its edges to target its application in the resolution of diseases. A proper potential application of this

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technology will bring a new revolution in biomedical research of recombinant DNA because, unlike previously known gene-editing tools, CRISPR-Cas9 is more efficient [4].

Studies previously conducted with CRISPR-Cas9 have shown tremendous advances in treating genetic mutations, typically those of single genetic locus displacement or replacement [6]. These studies used CRISPR with various Cas9 enzymes within human embryos [7] and cell culture [8]. Despite these advancements, studies of teratogenicity and mutagenicity in human cells are still undergoing multiple trials [9]. During previous experiments on gene therapy, scientists used a specific enzyme called 'restriction enzymes' to cut genes at a particular locus on the chromosome [10]. This resulted in a double-stranded break (DSB) in the DNA. Editing of the gene is done with specific tools, and the DSBs are repaired via two methods: non-homologous end joining (NHEJ) or homology-directed repair (HDR) [figure 1] [10]. However, CRISPR-Cas9 is an autonomous system encompassing various tools that replace the external application of restriction enzymes. This system presents multiple domains and a guide RNA that recognizes the target DNA region of interest within a biological system and directs the Cas-9 enzyme for editing [11].

## 2. Mechanism and Application of CRISPR-Cas9 Mediated Genome Editing in Sickle Cell Anaemia

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats – was remodelled with the Cas9 enzyme into a programmable genome-editing technique involving repetitive harvesting and collecting DNA sequences from the microbial genome [12]. The Cas9 enzyme within this sequence can cut DNA, prompting 'break-neck' research progress in the history of recombinant technologies [4]. The components of CRISPR-Cas9 are targeted explicitly for use in the recognition and cleavage of the genome during directive application. The first component of CRISPR is a single-effector Cas9 Enzyme, while the second component is a guide RNA (sgRNA) [13].

A recent study showed that with the application of CRISPR-Cas9 in solving related genetic problems during clinical therapies, there is a need for a compatible delivery system for the CRISPR-Cas9 tools in the biological system whether the ex-vivo and in-vivo genome editing is adopted [5]. This is a significant limitation because using compatible vectors is necessary for releasing an effective in-vivo human gene therapy [14]. In using CRISPR to solve sickle cell anaemia, there is a need for an immune-tolerant delivery system to aid perfection in precision and efficiency [15].

### 2.1. The Biological Delivery System for CRISPR-Cas9: Limitations, Repercussions, and a Way Forward

CRISPR-Cas9 delivery methods involve vectors (the mode of delivery into cells) and cargo (Cas9 nuclease and guide RNA) [16]. The delivery vectors fall into viral, non-viral, and physical categories. This vector is a significant determinant of the mode of translation of the Cas9 nuclease, whether as a DNA, mRNA, or protein [16].

### 2.2. Viral Delivery Vector, Pros and Cons

Adeno-associated viral vectors (AAVs), full-size adenoviral vectors (ATVs), and lentiviral vectors can all be used to administer CRISPR via viral delivery (LVs) [16]. Although this is a popular choice for in-vivo CRISPR investigations and clinical trials, it applies to ex-vivo and in-vitro studies [16]. They are notably more valuable in studies needing long-term expression, as in the case of correcting sickle cell anaemia [17].

Despite the immense value of applying this during clinical trials, significant side effects must be considered before using this delivery method so that CRISPR can be declared safe. As a side effect, the vector causes dangerous immune responses, leading to anaphylactic shock or the viral particle integrating its genetic material into the chromosome, leading to cancer [1]. The proposed biological delivery strategy for a programmed CRISPR-Cas9 is an adeno-associated viral vector [16]. This vector is commonly used for preclinical models and in-vivo delivery because the viral genome of the adeno-associated virus is not inserted into the host cell genome, and host immune responses are typically milder than when other viral delivery methods are used [18]. Notably, the use of this vector to treat certain diseases of similar lineage to sickle cell anaemia has been approved by the Food and Drug Administration (US) FDA [17]. However, this mode of delivery is a limited package because, unlike other viral vectors, these viruses are too small to accommodate both the Cas9 and sgRNA. Co-transfection by targeting sgRNA and Cas9 into a cell in separate AAVs with unique tags has successfully addressed this limitation [18].

### 2.3. Non-viral Vector, Pros and Cons

The non-viral vector mode of delivering CRISPR-Cas9 includes lipid-mediated delivery by membrane-derived vesicles [19]. This method is seemingly scientifically advanced but limited due to the immunogenic responses from antibody clusters formed against the Cas9 enzyme [19]. Recently, science has advanced toward the use of Nanoparticles in delivery. These tiny particles with a wide range of applications are designed with specific characteristics to target specific cell types for gene therapy [20]. They are less likely than viral vectors to cause immune reactions and are

easier to create and modify for applications [1]. Despite being a new procedure, the incorporation of lipid nanoparticles has proven helpful in delivering COVID-19 mRNA vaccines and might be an illustrator in the perfection of this procedure [21].

### 3. Application of CRISPR-Cas9: Targeting the Haemoglobin Beta-Chain, Procedures, and Results

The duration of sickle cell anaemia uses an ex-vivo technique with the expressive potential to correct the point mutation and substitute glutamic acid with Valine at position 6 of the haemoglobin beta-chain [22]. During an experimental practice, chemotherapy eliminated the remaining bone marrow to correct genetic flaws, allowing the repaired and re-infused stem cells to grow [23]. Daniel Dever, a research teacher in Matthew Porteus' group at Stanford University, devised a technique to extract Haematopoietic Stem Cells (HSCs) from a sickled patient [24]. He used a combination of CRISPR, rAAV6, and flow cytometry to induce single-nucleotide alterations into the genome of immunodeficiency mice; the HSC-targeting methodology and studies took about three weeks, and the long-term HSC engraftment analysis took about 16 weeks [24]. This procedure was carried out following troubleshooting and optimization procedures.

#### 3.1. Deploying the CRISPR-Cas9 Tools

In natural and manufactured CRISPR-Cas9 systems, the Cas9 enzyme finds and cleaves target DNA [25]. After deploying into a biological system, the sgRNA binds to the Cas9, inducing a conformational change that activates the Cas9 enzymes [25]. Since the target sickle cell anaemia is the haemoglobin beta-chain, there is a need to prevent off-target cleavage by carefully designating the CRISPR-Cas9 [26]. To this end, there is a need for proper modification and engineering of the sgRNA, which has shown tremendous progress in mitigating off-target effects [27]. Researchers are actively working on the experimental design of better models such as cytosine/adenine base editors, prime-editing, dCas9, Cas9 paired-nickase, and ribonucleoprotein (RNP) delivery [27]. After activating the Cas9 enzymes within the biological system, the Cas9 enzyme selectively edits the sickled genome on the haemoglobin chain, leveraging the synergism between its six domains (Figure 2) [25].

#### 3.2. Editing & Realignment

The protospacer adjacent motif (PAM)-Interacting domain inputs specificity and initiates binding of the programmed CRISPR-Cas9 to the target DNA within the stem cells [28]. In translation, the recognition (REC)-1 domain of the Cas-9 enzyme is the largest and responsible for binding the guide

RNA [28]. However, the Bridge-Helix kick-starts the cleavage activity upon binding target DNA [29].

The two endonuclease domains, 'HNH containing histidine and asparagine residues and RuvC, involved in DNA repair in *Escherichia coli*, cut the single-stranded DNA. These domains are highly homologous to endonucleases found in other proteins, such as those of the restriction enzymes [30]. Meanwhile, the RuvC cleaves the non-complementary DNA strand, while HNH cleaves the complementary strand. Together, these domains generate double-stranded breaks (DSBs) in the DNA of the haemoglobin beta chain, causing sickling [31].

#### 3.3. Cellular Repair of the Genome

Gene DSBs are repaired using two basic methods: gene knock-out and gene knock-in.

Gene Knock-In: This is achieved using homology-directed repair (HDR); the break site is exploited to insert a correction to the gene. The unedited DNA strand donor template contains the typical gene sequence used to fix the mutation by substituting glutamic acid with Valine at position 6 of the haemoglobin beta-chain. The altered cells are now modified to create normal haemoglobin and reimplanted in the patient's bloodstream when this fix is made [32]. Scientists and researchers can explore this pathway by providing an exogenous donor template with the CRISPR-Cas9 machinery to facilitate the desired genome editing [31].

Gene Knock-out: This is achieved using non-homologous end-joining (NHEJ), which involves random insertion and deletion of base pairs that code for glutamic acid at the cut site. However, this can lead to a frameshift mutation, resulting in a premature stop-codon or nonfunctional polypeptides [33]. Despite the limits, this route has proven to be particularly beneficial in genetic knock-out research and functional genomic CRISPR screenings; it is also helpful in the clinic when gene disruption gives a therapeutic opportunity. Remarkably, this technology has developed into a highly promising CRISPR-Cas9 sickle cell therapy procedure closer to clinical use than  $\beta$ -globin gene editing [33] by aiding the expression of haemoglobin F (HbF) enhanced by modifying (knocking out) the BCL11A gene [22]. This was after discovering that sickle cell patients with a natural mutation in their BCL11A gene were resistant to illness symptoms [22].

Once the HSC had been rectified, these stem cells were reimplanted into bone marrow and subjected to chemotherapy [24], triggering new haemoglobin in the form of HbF. These reverse sickling and curatively affect sickling cell disease in a proposed adult [33].

### 4. Conclusion: CRISPR-Cas9, a Structure for the Future

Various questions exist within science on the safety of the CRISPR-Cas9 technique [34], challenges of bioethical concerns are noted on solid grounds, and researchers are ad-

vancing a resolution to all limitations of this technique [34]. An experiment in human embryos revealed that CRISPR could resolve a defective MYBPC3 allele that causes cardiomyopathies in humans [7]; another trial showed that CRISPR-Cas9 can ex-vivo genome-editing therapy for hemoglobinopathies [35]. However, Nigeria is an endemic area for associated genetic diseases and has no evident research or trials regarding CRISPR-Cas9. Frankly, such techniques have never been practiced [36]. A method such as CRISPR-Cas9 will give many people hope and help abolish the disease in sufferers. This will prevent the yearly mortality rate increase from sickle cell diseases and other haemoglobinopathies [36].

The investigation of CRISPR continues as science and technology advance, and I hope and wish that someday, this technology will become a reality in solving the problems of my people (Nigerians and Africans). Significant feats have been recorded in the experimental and clinical application of CRISPR-Cas9 [9]. Even with the high prevalence of risks associated with CRISPR-cas9, research is ongoing to minimize these risks. Research is ongoing; science is advancing, and one day, this hope will become an achieved reality.

### Abbreviations

- NHEJ Non-Homologous End Joining
- HDR Homology-Directed Repair

### Appendix

- AAVs Adeno-Associated Viral Vectors
- ATVs Adenoviral Vectors
- LVs Lentiviral Vectors
- HSCs Haemopoietic Stem Cells
- DSB Double-Stranded Break
- PAM Protospacer Adjacent Motif
- RNP Ribonucleoprotein

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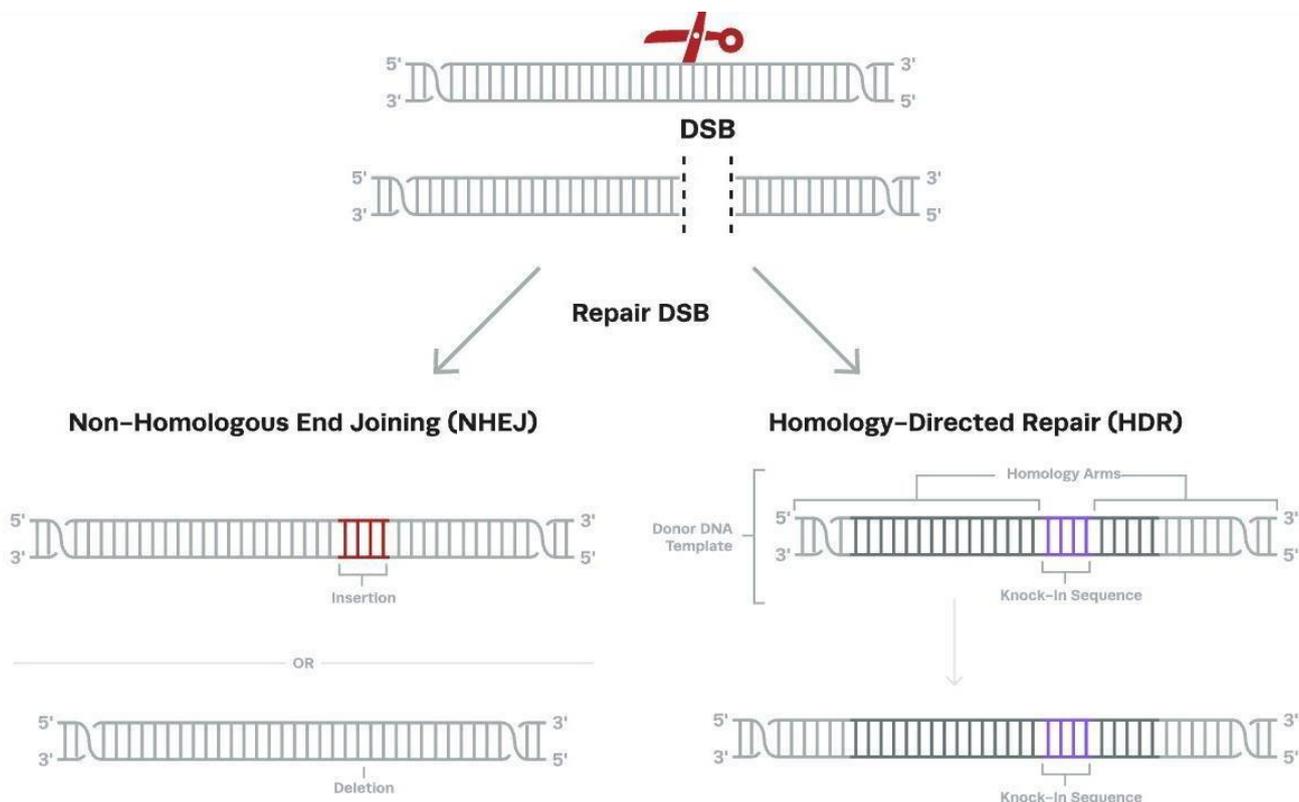
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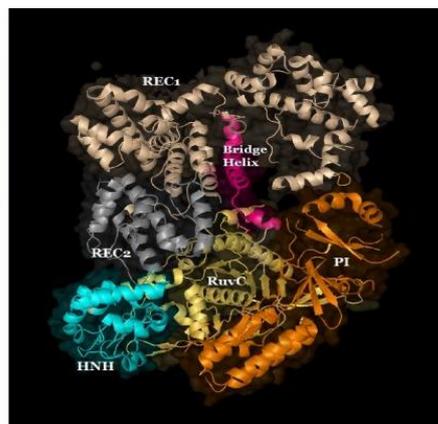
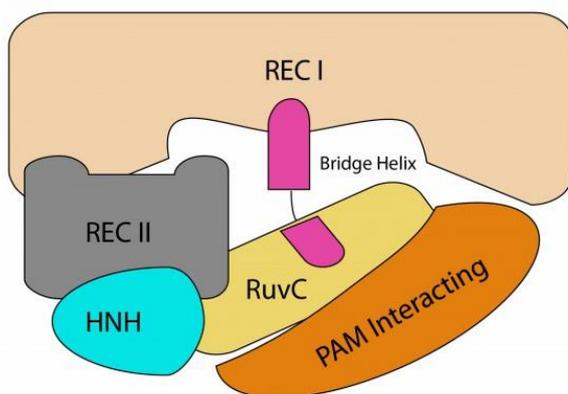
### Author Contributions

Babatunde Olowu conceptualized the manuscript and drafted the first edition, while all other authors worked on modeling the manuscript till the final version. The authors have read and approved the final manuscript.

### Conflicts of Interest

The authors declare no conflicts of interest.



**Figure 1.** How Genome Editing is Accomplished Source: The Bench.**Figure 2.** Cas9 Enzyme. The Cas9 enzyme comprises Rec I, Rec II, Bridge Helix, RuvC, HNH, and PAM Interacting domains—source: Tufts.edu.

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## Biography



**Babatunde Olowu** is a final-year veterinary student from the University of Ibadan with a budding interest in biomedical research across the Immunology, Vaccinology, and Molecular pathogenesis of Infectious diseases. He was one of the participants in the 2022 Veterinary Research Scholars program (V.R.S.P.) at the University of Missouri, Columbia, U.S.A. Also, he participated in the 2023 Boehringer Ingelheim Award research program at Kansas State University, Manhattan, U.S.A. Babatunde is a recipient of the annual Bovas Scholarship, the 2022 Raymond Zard Foundation scholarship, and was also awarded the MSD Animal Health/World Veterinary Association (WVA/MSD) Veterinary Student Scholarship Award in 2024. Babatunde's has won several grants, with proposal approval from organisations like Morris Animal Foundation and the Washington State University C.V.M.