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Progress on CRISPR -Cas9 Gene Editing Technology in Sickle cell disease: A Review

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ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system as a method of Gene editing possess a broad range of applications for genetic modification, diagnosis and treatment for curing non-infectious (such as the Sickle cell disease) as well as infectious diseases. Initially identified in bacteria and archaea to fight plasmids' DNA and/or bacteriophage infecting bacteria. Cas9 and gRNA forms a complex to target and cleave the desired gene, providing defense (adaptive immunity) against viral infections to the host, also allow bacteria to recognize genetic sequences using specialized enzymes or CRISPR-associated proteins (Cas), including the DNA endonuclease, Cas9 systems (Molecular scissors). In this review we focus on the use of CRISPR/Cas9 gene-editing for curing Sickle cell disease (SCD), including the curative correction of SCD mutation in β-globin (HBB) and the induction of fetal hemoglobin to reverse sickling, that culminates with Casgevy and Lyfgenia Gene therapy approval by US Food and Drug Administration and UK Medicines and Healthcare Products Regulatory Agency. We summarize the major achievements and challenges, aiming to provide a clearer perspective on the potential of gene-editing based approaches in curing SCD using Prime editors to minimize off-target effect, cellular toxicity of delivery systems and durability as well as making it accessible and affordable to the low-income countries with highest prevalence in sickle cell disease.

Keywords: *CRISPR -CAS9, Gene Editing Technology, Sickle cell disease, Cure affordability*

INTRODUCTION

In 2020, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for their research on the endonuclease (Molecular scissors), clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated protein 9 (CRISPR-Cas9) method for DNA editing.[1]

November, 2023, the UK Medicines and Healthcare Products Regulatory Agency (MHRA) was the first to approve the CRISPR-Cas9 gene editing therapy, Casgevy (exagamglogene autotemcel), for the treatment of patients with transfusion-dependent β-thalassemia and the treatment of sickle cell disease in patients aged ≥12 years with recurrent vaso-occlusive crises. By December of the same year the US Food and Drug Administration (FDA) approved both Casgevy and Lyfgenia (lovotibeglogene autotemcel) for patients with sickle cell disease. Both Casgevy and

Lyfgenia were the first clinical therapeutic applications of CRISPR-CAS9 that begins with studies on Sickle cell disease and beta-thalassemia.[2]

Sickle cell disease (SCD) is the most common monogenic hematologic disorder and is essentially congenital hemolytic anemia caused by an inherited point mutation in the β-globin on chromosome 11. Although the genetic basis of SCD was revealed as early as 1957, treatment options for SCD have been very limited. Hematopoietic stem cell transplantation (HSCT) was thought to hold promise as a cure for SCD, but the available donors were still only 15% useful. Gene therapy has advanced rapidly into the 21st century with the promise of a cure for SCD, and gene editing strategies based on the cluster-based regularly interspaced short palindromic repeat sequence (CRISPR)/Cas9 system have revolutionized the field of gene therapy by precisely targeting genes.[3]

The most studied endonucleases, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated protein 9 (CRISPR-Cas9) system is one of the Gene editing technique used to treat genetic diseases like Sickle cell anemia, Cystic fibrosis, Duchenne Muscular dystrophy etc. initially identified in bacteria to fight bacteriophage infection, also allow bacteria to recognize genetic sequences using specialized enzymes or CRISPR-associated proteins (Cas), including the DNA endonuclease, Cas9 .

Other techniques like TALENs (Transcription Activator-Like Effector Nucleases), ZNFs (Zinc Finger Nucleases), Base Editing, Prime Editing are used for Gene therapy approaches Ex vivo, In vivo including the controversial Germline (with ethical approval) to achieve Gene correction, Gene disruption and/or Gene regulations, making a PRECISE changes to DNA sequence of an individual at the mutated point (s), modifying it (like in the case of HbS). [3,4]

Gene editing uses an endonuclease to cut a region of DNA and bring the two cut ends together or insert a new or altered gene sequence.

In contrast to conventional gene therapy approaches, gene-editing technologies (CRISPR-CAS9 SYSTEM) offer the potential to permanently modify disease-causing genes through precise correction, deletion, addition, and/or disruption of specific sequences.[3]

HEMOGLOBINOPATHIES

There are several clinical subgroups of sickle cell disease (SCD), including hemoglobin SC disease (HbSC), sickle cell anemia (SCA), and hemoglobin sickle-β-thalassemia (β-thalassemia-positive or β-thalassemia-negative), with other minor variants. Patients with the sickle cell trait (HbAS) who carry a heterozygous gene mutation do not usually have clinical signs or symptoms [8,9].

Thalassemia is a common cause of microcytic anemia due to impaired synthesis of hemoglobin globin protein [10]. β-thalassemia is an inherited mutation of the β-globin gene on chromosome 11, causing a reduced β-globin chain of hemoglobin, and occurs most commonly in people of Asian, Mediterranean, and Middle Eastern origin [10,11]. The prevalence of β-thalassemia is between 80–90 million carriers and up to 1.5% of affected individuals in the global population [8]. Patients have varied genotypic and phenotypic presentations because more than 200 mutations have been identified in the β-globin gene [12]. There are three classifications of β-thalassemia: β-thalassemia minor (carrier or trait) is heterozygous and usually asymptomatic with mild anemia; homozygous or compound heterozygous β-thalassemia mutations result in a more severe range of anemias called β-thalassemia intermedia and β-thalassemia major, which are distinguished clinically by transfusion non-dependence or dependence [13].

CRISPR-CAS9 TECHNOLOGY APPLICATIONS IN SCD

Sickle cell disease and β-thalassemia have become the paradigm for CRISPR-based therapeutic genome editing in human disease, with the first regulatory approvals for Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel) [14]. These gene editing therapies use the patient's hematopoietic stem cells, modified ex vivo by genome editing using CRISPR-Cas9 technology, and then transplanted as a single-dose infusion [19,20]. Before treatment, the patient's stem cells are collected and undergo myeloablative conditioning with high-dose chemotherapy. The modified stem cells are then transplanted to the patient, engrafting within the bone marrow [7,15]. Casgevy (exagamglogene autotemcel) is used to edit CD34+ cell human hematopoietic stem and progenitor cells at the erythroid-specific enhancer region of the BCL11A gene, which prevents the production of fetal hemoglobin (HbF). Lyfgenia (lovotibeglogene autotemcel) is a cell-based gene therapy that uses a lentiviral vector with blood stem cells genetically modified to produce HbAT87Q, which functions in a similar way to normal adult hemoglobin (HbA) to reduce the risk of red blood cell sickling and to improve vascular blood flow [16].

Several gene editing strategies for curing SCD have shown promise in recent preclinical studies, including: (i) correction of the causative point mutation in HBB, (ii) induction of fetal hemoglobin (HbF) viagene-disruption of γ-globin (HBG) repressors, and (iii) induction of HbF via introducing beneficial hereditary persistence of fetal hemoglobin (HPFH) mutations on the β-globin locus [17].

HbF induction can be achieved by silencing transcription factors such as B-cell lymphoma/leukemia 11A (BCL11A) that mediate silencing of HBG after birth [19] or mimicking beneficial HPFH mutations [39]. In addition, the identification of novel HbF regulators is an active area of research [20].

BCL11A is a transc-ription factor that represses γ-globin expression and fetalhemoglobin in erythroid cells

Sickle cell disease symptoms appear in infancy as γ-globin gene (HBG1 and HBG2) transcription switches to β-globin (HBB), causing a shift from fetal hemoglobin (HbF; α2γ2) to HbA in RBCs.

Around the time of birth, the site of red blood cell production shifts from the fetal liver to the bone marrow, and this transition is associated with a switch from γ-globin (HBG1/HBG2) to β-globin (HBB) production.2 Consequently, HbF declines and HbA (or HbS in case of individuals with sickle cell disease) increases. The γ-to-β-globin switch is an intriguing paradigm of the developmental regulation of gene expression and is clinically important because β-hemoglobinopathies can be treated by inhibiting this switch. 2 This perinatal γ-globin–to–β-globin switch is mediated by transcriptional repressor proteins, BCL11A and ZBTB7A/LRF, that bind cognate cis-regulatory elements in the HBG1/HBG2promoters.14 Inhibiting the binding of these repressors to their targets in adult RBC precursors can reactivate expression of γ-globin and HbF.14 Transduction of sickle cell disease patient hematopoietic stem cells (HSCs) with a lentiviral vector encoding an erythroid-expressed short hairpin RNA against BCL11A,10 or targeted disruption of a BCL11A erythroid-specific enhancer by the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 nuclease system followed by autologous HSCT,11 resulted in elevated HbF and reduced sickle cell disease symptoms. Additional strategies to increase HbF through genetic manipulation of HSCs also exist.2,13 It is not yet known which strategies are the safest and most effective for clinical application [21].

2. Genetic modification of autologous HSCs shows great promise for treating sickle cell disease, although the best approaches are not yet established. Lentiviral vector–

mediated addition of an anti-sickling β-like globin is effective, 20 although this approach does not eliminate βS-globin expression and creates a potentially toxic excess of β-like globin chains which can lead to ineffective erythropoiesis and erythroid dysplasia.21 In contrast, induction of endogenous γ-globin transcription concomitantly reduces βS-globin, thereby maintaining the balance of α-globin and β-like globin chains.

3. Although the off-target effect remains a potential issue, it can be significantly reduced by rational gRNA designs or utilizing high-fidelity Cas9 protein. Base editors are created by fusing a nucleotide deaminase with catalytically disabled Cas9 protein. Base editors directly convert one base into another without inducing DSBs and therefore not relying on HDR, enabling the point mutation correction in non-dividing cells. Therefore, base editors are a promising DNA editing tool and considered to be preferable to using Cas9 nuclease which may lead to the generation of unwanted small insertions/deletions (indels), translocations, or chromosomal rearrangements [23]

With the advancement of gene-editing technologies, each of the four technologies (ZFNs, TALENs, CRISPR/Cas9, base editor) have been tested in HSPCs for treating SCD.

MATERIALS & METHODS

A literature search was done from July, 2024 to October 2024 with restrictions to the English language. The search was performed in ScienceDirect, PubMed, Elsevier and from reliable online news sources, using a combination of search terms related to Gene therapy, Gene Editing, CRISPR-CAS9 system, TALENs, Zinc Finger Nucleases ZNFs, Molecular scissors, Endonucleases, Sickle cell anemia/disease, Hemoglobinopathies, Prime Editing, Base editors, Single point polymorphism SNPs, healthcare, affordability. The initial search yielded a total of more than 220 relevant publications, from which 20 were carried forward for a title and abstract screening. Five articles were selected for systematic synthesis. Among the reasons for exclusion were irrelevance to Sickle cell disease application for the technology, healthcare purpose, and irrelevance to genetic mutations applications. An updated search was done time to time. Overall, three studies assessed CRISPR-Cas9 and one study used Mouse model to edit genome. The use of gene editing solution to single nucleotide polymorphism problems associated with the disease of blood shows a promising cure to the burden of sickle cell disease with an immense importance.

RESULT

IN VIVO APPROACH

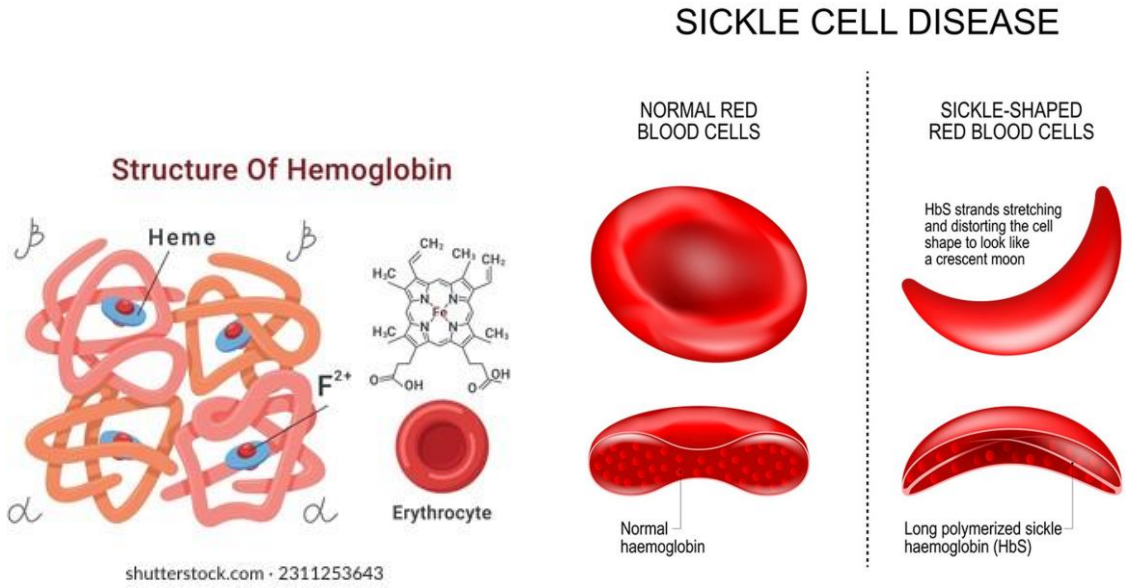
The ex vivo strategies described above are, however, complex, which involve harvesting HSCs through leukapheresis or bone marrow (BM) aspiration, myeloablation by chemotherapy, in vitro HSC culture, and transplantation.

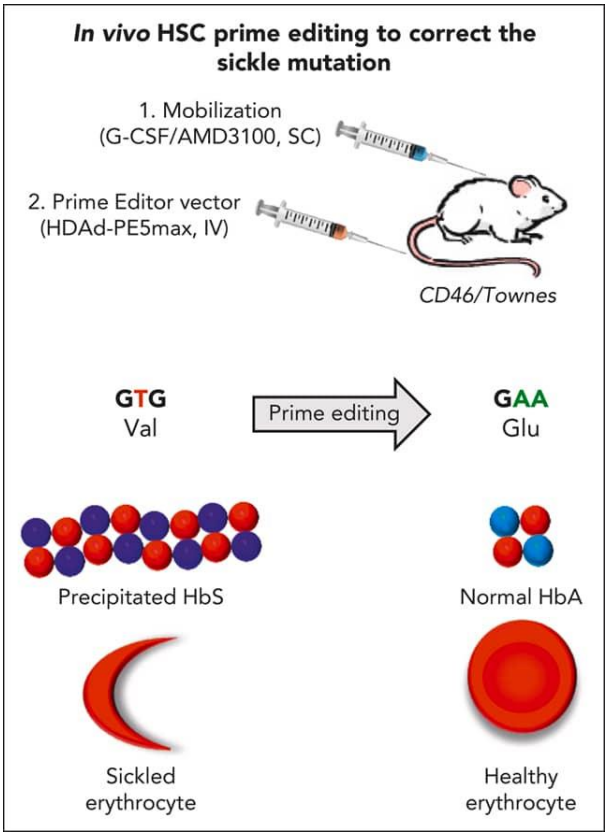
A vectorized prime editing system that can directly repair the SCD mutation in hematopoietic stem cells (HSCs) in vivo in a SCD mouse model (CD46/Townes mice) was described by researchers recently. Because of its simplicity and portability, the in vivo prime editing approach has the potential for application in resource-poor countries where SCD is prevalent.

The most desirable strategy for genetic correction of SCD is to convert the pathogenic codon (valine, GTG) to the wild-type (glutamic acid, GAG). Recently developed prime editors (PEs) can catalyze the T>A conversion. PEs contain a catalytically impaired SpCas9 nickase (nCas9) fused to an engineered reverse transcriptase (RT) that directly copies edited sequence information from a prime editing guide RNA (pegRNA) into a target DNA locus. This causes the cell to replace the original DNA sequence on both strands with the newly synthesized DNA flap.

Because the PE system involves 3 separate DNA binding events between (1) the guide

sequence and the target DNA, (2) the primer binding site and the target DNA, and (3) the 3' end of the nicked DNA strand and the pegRNA, it has been suggested to have fewer undesirable off-target (OT) effects than CRISPR/Cas9.5, 6, 7. Furthermore, in contrast to CRISPR/Cas9, PEs are independent of double-strand DNA breaks, therefore minimizing the generation of unwanted random insertions and deletions (indels) at the target site and large genomic rearrangements or translocations.8 Recent improvement in the PE architecture,9 the pegRNAs,10 and the inclusion of an engineered dominant-negative MLH1 gene (e.g., PE5max) resulted in efficient target site editing and fewer undesired indels[23]





Mouse model for Single Nucleotide Polymorphisms in SCD

The in-vivo cure affordability depiction that will help in bringing the much needed technology to the low income county fast

DISCUSSION

CHALLENGES AND WAY FORWARD

One of the significant challenges in utilizing CRISPR-Cas9 for treating SCD is ensuring that the system specifically targets only the mutated gene without affecting other genes in the genome. The potential for off target effects that can lead to unintended changes in DNA, is posing a significant safety concerns.

The delivery systems' efficiency and selectivity with minimal toxicity to other cells. Additionally, the efficiency of the CRISPR-Cas9 system to be high enough in achieving a therapeutic effect couple with the durability are the important factors considered in many studied in CRISPR-CAS-based-genetic editing technology, unfortunately, the cost of developing and commercializing CRISPR-based therapies are very high, making them prohibitively expensive for many patients, particularly those in low-income countries where the prevalence is high. There is a need to ensure that CRISPR-based therapies are accessible and affordable to all patients who could benefit from them[24].

Li and colleagues report on a novel gene-therapy approach to sickle cell disease. Rather than ex vivo manipulation with lentiviral gene addition or CRISPR/Cas9-mediated fetal hemoglobin reactivation, the authors describe injection of a nonintegrating prime editor–expressing vector into a sickle mouse model with

correction of over 40% of hemoglobin S alleles in vivo [25].

The in vivo approach is technically simple; it only involves subcutaneous injections to mobilize HSCs, an intravenous injection of a single nonintegrating HDAd-PE5max vector, and expansion of corrected HSPCs by early treatment with low-dose.

We believe that the in vivo prime editing approach to correct the SCD mutation, described in their article, could be the basis for further development toward a cost-effective SCD gene therapy that could be used in developing countries that requires it more.

CONCLUSION

SCD poses a significant financial burden on patients' families and society, resulting in extensive hospitalizations, readmissions, emergency department (ED) visits, and other costs associated with the healthcare system. Although measures such as newborn screening and related complications management have partially improved SCD prognosis, the average life expectancy of patients is only 54 years [26]. Therefore, extending the lifespan and improving the quality of survival for SCD patients is an urgent issue that needs to be addressed with the help of advancing medicine and biotechnology to get an accessible and affordable cure. Hematopoietic stem and progenitor cells (HSPCs) are a rare cell population capable of self-renewal and multipotent differentiation to all hematopoietic lineages and are crucial in the maintenance of lifelong production of all blood cells. Thus, successful gene addition or correction in a relatively small number of HSCs can translate into clinically meaningful levels of RBC chimerism in peripheral blood. These special properties of HSPCs have fostered their widespread use in cell and gene therapy. This knowledge has resulted in dramatic progress in treating inherited hematopoietic diseases including the Sickle cell anemia that involves adding a segment of a normal β-globin and correcting or inactivating a mutated β-globin in the patient's cells. Gene-modified HSPCs can generate healthy erythrocytes with normal morphology and extended lifespan, unlike sickle erythrocytes, but the cost of the treatment is making it prohibitively accessible to many patients, hence the need for an in-vivo prime editing incorporation.

Declaration by Authors

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