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Targeting BCL11A with CRISPR-Cas9 Gene Editing Technology to Treat Sickle Cell Disease

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Abstract. An enormous portion of people from all over the world are afflicted by sickle cell disease (SCD), which is a hereditary blood malady that has a severe negative influence on quality of life. Despite advancements in supportive care, a definitive cure remains elusive. The transcriptional repressor BCL11A has surfaced as an enticing target for novel SCD therapies. The prospect of applying CRISPR-Cas9 gene editing to alter BCL11A promoter and optimize SCD pathogenesis is examined in this paper. By suppressing BCL11A, which normally inhibits fetal hemoglobin (HbF) expression, HbF levels can be elevated in adult red blood cells. Increased HbF mitigates the deleterious effects of sickle hemoglobin polymerization, the root cause of SCD manifestations. Preclinical investigations deploying CRISPR-Cas9 to disrupt BCL11A in hematopoietic stem cells have yielded promising results, paving the way for pioneering clinical trials. Although in its early stages of development, this gene editing method has great potential as a revolutionary, one-time therapeutic intervention for SCD. As research progresses, meticulous evaluation of safety, efficacy, and long-term outcomes can be paramount in translating this groundbreaking strategy into clinical reality.

Keywords: Sickle cell anemia, BCL11A repressor, CRISPR-Cas9 gene editing, fetal hemoglobin induction.

1. Introduction

A legion of individuals worldwide is affected by sickle cell anemia, a debilitating genetic blood illness that affects over 300,000 newborns annually.[1]. It is particularly prevalent in sub-Saharan Africa, where it poses a significant public health burden [2]. A single-point mutation in the β-globin gene results in sickle cell disease (SCD) by producing aberrant hemoglobin (HbS). When oxygen levels are low, hemoglobin polymerizes, causing red blood cells to swell into a sickle shape. These sickled cells have the ability to block blood arteries, which can lead to hemolytic anemia, vaso-occlusion, and gradual damage to several organs [1]. SCD patients often experience recurrent painful crises, acute chest syndrome, stroke, and other life-threatening complications. Even with improvements in supportive care, people with SCD still have a 20–30 years shorter life expectancy than people without the disease.

Hemoglobin S (HbS) undergoes polymerization under low oxygen conditions, leading to the repeated deformation and destruction of red blood cells (RBCs). These malformed RBCs adhere to vascular endothelium through interactions with white blood cells and platelets mediated by adhesion molecules, causing blockages in blood vessels. The breakdown of RBCs releases free hemoglobin and heme, which consume nitric oxide and cause endothelial cells to function improperly, leading to further narrowing of

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vessels. This exacerbates vaso-occlusion and triggers inflammatory reactions marked by elevated levels of cytokines and oxidative stress, thereby promoting more blockages in the vessels [1].

BCL11A is a transcriptional factor. Currently, targeting BCL11A has been used in the treatment of SCD [3,4]. BCL11A represses the expression of γ-globin, which is a component of fetal hemoglobin (HbF). During fetal development, adult hemoglobin (HbA) eventually replaces the main hemoglobin, HbF, after birth. On the other hand, those who have spontaneous mutations that affect BCL11A function or interfere with its erythroid-specific enhancer show continuous HbF expression throughout their lives [4]. Importantly, these people with fetal hemoglobin persistence hereditary (HPFH) have milder SCD symptoms and improved clinical outcomes. Therefore, silencing BCL11A to reactivate HbF production in adult erythrocytes has become an attractive strategy for SCD treatment [5].

Gene therapy has undergone a revolutionary change with the advent of CRISPR-Cas9 gene editing technology, which has also created novel options for therapy for SCD. CRISPR-Cas9 enables precise and efficient targeting of the BCL11A erythroid enhancer in hematopoietic stem cells (HSCs) [3]. γ-globin derepression and HbF induction result from particular reductions in BCL11A expression in erythroid cells by disruption of this enhancer. For patients with SCD, autologous transplantation of these modified HSCs could serve as a single, effective treatment [3,6]. Early clinical data from ongoing trials has shown promising results, with stable engraftment of edited cells, increased HbF levels, and reduced SCD-related complications in treated patients [3].

This review summarizes BCL11A's role in controlling hemoglobin production and how it could be a promising target for treating SCD. The review summarizes the early development of making use of CRISPR-Cas9 for boosting HbF levels by editing the BCL11A enhancer and discusses about the first clinical results in SCD patients. Moreover, this review points out how BCL11A is regulated differently between species and explores future directions for improving this gene editing approach to make it available to more people with SCD.

2. CRISPR-Cas9 gene editing technology

CRISPR-Cas9 is a novel approach to gene editing, inspired by immune systems that adapt to bacteria and archaea [3, 4]. Its two primary components are a single guide RNA (sgRNA) and Cas9 endonuclease. Using the sgRNA as molecular scissors, the Cas9 nuclease may cut DNA at a designated site. Synthetic RNA molecules, or sgRNAs, are designed to guide Cas9 to the appropriate chromosomal site. They have a complementary 20-nucleotide sequence to the target DNA [7]. Subsequently, the natural DNA repair machinery of the cell attempts to repair the break, usually via the erroneous non-homologous end joining (NHEJ) route. NHEJ often results in small insertions or deletions (indels) close to the cut site, which may impair the target gene's functionality [4].

For BCL11A inhibition in SCD, CRISPR-Cas9 is designed to specifically target the erythroid-specific enhancer located in the second intron of the BCL11A gene. This enhancer is critical for the expression of BCL11A in erythroid cells, but not in other cell types [4]. By deleting or mutating this enhancer, BCL11A expression is selectively reduced in red blood cells, leading to the reactivation of fetal hemoglobin (HbF) without affecting BCL11A's important functions in other tissues [2,4].

Several research groups have developed sgRNAs that efficiently target the BCL11A erythroid enhancer and have showed operational editing in human hematopoietic stem and progenitor cells (HSPCs)[1, 2].Pre-assembled Cas9-sgRNA ribonucleoprotein (RNP) complexes have been successfully delivered by electroporation, which has been shown to produce considerable amounts of biallelic enhancer deletion [3] Importantly, the edited HSPCs maintain their long-term repopulating capacity and differentiate into erythroid cells with elevated HbF levels, highlighting their therapeutic potential for SCD [4,5].

In addition to the *ex vivo* editing of HSPCs, alternative CRISPR-Cas9 delivery methods are being explored for *in vivo* BCL11A targeting. These include the use of adeno-associated virus (AAV) vectors to deliver Cas9 and sgRNA components directly to the bone marrow [9]. While still in early stages, *in vivo* gene editing could potentially simplify the treatment process and expand patient access.

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As CRISPR-Cas9 technology continues to evolve, novel variants of Cas9 with improved specificity and efficiency are being developed [3]. Furthermore, the targeting of BCL11A may be improved and the likelihood of unintentional mutations decreased by using base editors and prime editors, which allow for precise single-nucleotide modifications or minor insertions without generating DSBs [4]. These advancements in CRISPR-Cas9 technology hold great promise for advancing SCD gene therapy.

3. Applications of CRISPR-Cas9 on SCD

Early-stage and clinical studies on SCD have made use of CRISPR-Cas9 gene engineering technologies. Table 1 summarizes the models, genes targeted, particular techniques, and outcomes of CRISPR-Cas9 gene engineering strategies in sickle cell anemia.

3.1. Preclinical studies

A lot of studies in cells and animal models have shown that using CRISPR-Cas9 to edit the BCL11A enhancer in human blood stem cells works well. Canver et al. used Cas9 and sgRNA to delete parts of the enhancer, which increased HbF to over 80% in red blood cells grown from edited SCD stem cells [5]. The edited cells could still turn into different types of blood cells and didn't have any unintended mutations. Similarly, Wu et al. showed that getting rid of the BCL11A enhancer using Cas9 in SCD stem cells led to over 60% HbF and better blood test results when transplanted into mice with weak immune systems [6]. These early results set the stage for testing this approach in human patients.

3.2. Clinical trials

The CLIMB trials (NCT03745287) are the first-in-human studies to evaluate the security and effectiveness of CRISPR-Cas9 BCL11A enhancer editing in SCD patients [7]. In these trials, autologous HSPCs are harvested from patients, edited *ex vivo* using Cas9-sgRNA RNPs, and re-infused after myeloablative conditioning. Preliminary data from the trials showed that the gene-edited HSPCs engrafted successfully and resulted in durable HbF elevation to 40-50% in treated patients at 6-18 months follow-up [7]. Total hemoglobin levels rose to 11-15 g/dL, and no vaso-occlusive episodes were observed post-treatment. These promising early results suggest that BCL11A enhancer editing could provide significant clinical benefits and potentially a functional cure for SCD patients.

3.3. Long-term safety and efficacy monitoring

While the initial clinical data is encouraging, longer-term safety and efficacy monitoring is crucial to fully assess the impact of CRISPR-Cas9 BCL11A editing in SCD patients. The CLIMB trials include a 2-year follow-up period to evaluate the durability of HbF induction, hematological improvements, and overall clinical outcomes [7]. Additionally, patients will be enrolled in a long-term follow-up study for 15 years post-treatment to monitor for any delayed adverse events or changes in treatment efficacy over time. Thorough safety evaluations, including surveillance for deviation editing, insertional genetic mutations, and possible immunogenicity, will be executed to guarantee the sustained safety of the methodology.

3.4. Optimization of gene editing protocols

Ongoing research aims to further optimize the CRISPR-Cas9 BCL11A editing protocols to enhance editing efficiency, minimize off-target effects, and improve overall safety. Novel Cas9 variants with increased specificity, such as high-fidelity Cas9 (HiFi Cas9) and enhanced specificity Cas9 (eSpCas9), are being explored to reduce off-target editing [8]. Further, in an effort to increase the effectiveness and targetability of the gene editing components, several delivery strategies including adeno-associated virus (AAV) vectors or nanoparticle-based systems are being researched [9].

3.5. Non-deletional enhancer disruption methods

While targeted deletion of the BCL11A erythroid enhancer has shown promising results, concerns remain regarding the potential consequences of introducing double-strand breaks (DSBs) and the risk

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of off-target editing. To address these concerns, non-deletional enhancer disruption methods, such as base editing and prime editing, are being explored [4]. Base editing enables precise single-nucleotide modifications without inducing DSBs, potentially mitigating off-target effects. Without the need for donor templates or DSBs, prime editing, a combination of Cas9 nickase and reverse transcriptase which enables precise insertions, deletions, and base conversions [3]. These alternative approaches may provide safer and more precise options for BCL11A enhancer disruption in the future.

3.6. In vivo gene editing strategies

Whereas the outcomes of *ex vivo* gene editing of autologous HSPCs have been encouraging, the creation of *in vivo* gene editing techniques has the potential to completely change the way that SCD is treated.

In vivo delivery of CRISPR-Cas9 components directly to the bone marrow or other hematopoietic tissues could potentially eliminate the need for HSPC harvesting, *ex vivo* manipulation, and transplantation [8,9]. This approach would simplify the treatment process, reduce procedural risks, and expand patient access. However, significant challenges, such as ensuring targeted delivery, achieving sufficient editing productivity, and minimizing undesirable consequences, need to be addressed before *in vivo* gene editing can become an appealing choice for SCD treatment.

Table 1. Applications of CRISPER-Cas9 gene editing strategies in SCD

Models or trails	Targeting	Methods	Results	Ref(s)
SCD stem cell	The BCL11A enhancer	Use and sgRNA to delete parts of the enhancer	HbF increase to over 80% in red blood cells	[5]
CLIMB trials (NCT03745287)	The BCL11A enhancer	Collect autologous HSPCs from patients, edit *ex vivo* using Cas9-sgRNA RNPs, re-infused after myeloablative conditioning	HbF elevation to 40-50% and total hemoglobin levels rise to 11-15 g/dL,	[7]
Ex vivo gene editing of autologous HSPCs	HSPCs	CRISPR-Cas9 components directly to the bone marrow or other hematopoietic tissues	Simplify the treatment process, reduce procedural risks, and expand patient access	[8,9]

4. Conclusion

CRISPR-Cas9 gene engineering has revolutionized the area of genetic therapies, particularly for SCD. The effectiveness and therapeutic potential of targeting the BCL11A erythroid enhancer to revive the synthesis of fetal hemoglobin (HbF) have been proven by preclinical and early clinical studies. Future and ongoing clinical trials will assess this approach's long-term safety, durability, and patient results. To optimize the gene editing protocol, researchers are exploring novel Cas9 variants, alternative delivery methods, and non-deletional enhancer disruption techniques such as base editing, which may improve editing efficiency and safety and minimize off-target concerns associated with double-strand breaks (DSBs). Additionally, initiatives are being made to create *in vivo* gene editing techniques, which may streamline the therapeutic regimen and increase patient accessibility. As this technology progresses, it is crucial to address ethical considerations, ensure equitable access, and provide comprehensive patient education and support to maximize the benefits of this innovative approach for the global SCD community.

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