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Application and Breakthrough of CRISPR-Cas9 Gene Editing Technology in the Clinical Treatment of Sickle Cell Disease

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Abstract:

Sickle Cell Disease (SCD) is an inherited hemoglobinopathy caused by a point mutation (GAG→GTG) at the 6th codon of the β-globin gene (HBB). Among traditional treatments, hydroxyurea requires lifelong administration and some patients show poor response; although hematopoietic stem cell transplantation (HSCT) can cure the disease, only 18% of patients can find HLA-matched donors, and there is also the risk of graft-versus-host disease (GVHD). The CRISPR-Cas9 gene editing technology provides a potential curative option for SCD by precisely modifying hematopoietic stem/progenitor cells (HSPCs). This article systematically reviews the clinical application strategies, experimental evidence, and existing challenges of this technology, and discusses its translational prospects in combination with the latest research findings. It covers the clinical effects of strategies such as targeting the BCL11A gene enhancer to activate fetal hemoglobin (HbF), directly correcting the E6V mutation of the HBB gene via homology-directed repair (HDR), and mimicking hereditary persistence of fetal hemoglobin (HPFH), as well as safety and accessibility challenges (e.g., off-target effects, preconditioning toxicity, and high treatment costs) and their corresponding solutions, aiming to provide comprehensive references for the research and application of gene therapy in SCD.

Keywords: Sickle cell disease; CRISPR-Cas9; gene editing; fetal hemoglobin; clinical treatment.

1. Introduction

As one of the most common monogenic inherited diseases worldwide, Sickle Cell Disease (SCD) exhibits significant geographical differences in its epidemiological characteristics. Statistics show that there are approximately 100,000 patients in the United States, while over 3 million patients in sub-Saharan Africa, with 50%-90% of affected children not surviving beyond the age of 5. The disease is caused by a point

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mutation in the 6th codon of the HBB gene (GAG→GTG), which leads to the polymerization of abnormal HbS under deoxygenated conditions, resulting in sickling of red blood cells and triggering vaso-occlusive crisis (VOC), chronic hemolytic anemia, and multi-organ damage [1].

However, traditional treatment regimens have obvious limitations. Although hydroxyurea can induce the expression of fetal hemoglobin (HbF), it requires lifelong administration and some patients show poor response. HSCT, as the only curative method, is only accessible to 18% of patients who can find HLA-matched donors, and there is a risk of graft-versus-host disease (GVHD). In 2023, the U.S. FDA approved the CRISPR-Cas9 therapy Casgevy (exagamglogene autotemcel), which activates HbF expression by editing the BCL11A gene enhancer, bringing a revolutionary breakthrough to SCD treatment [2].

Existing studies have confirmed the multi-faceted applications of CRISPR-Cas9. For example, strategies such as direct repair of HBB mutations, targeting BCL11A or γ-globin promoters have all shown effects in reducing the incidence of VOC and increasing HbF levels in clinical trials. This article will systematically analyze the current status and future trends of CRISPR-Cas9 in the treatment of SCD, and elaborate on it specifically from aspects including the core mechanism of action of the technology, the empirical evidence accumulated from clinical applications, safety assurance measures, and the ethical controversies and challenges it faces.

2. Molecular Mechanisms and Strategies of CRISPR-Cas9 in SCD Treatment

2.1 Targeting the BCL11A Gene Enhancer to Activate HbF

When the CRISPR-Cas9 is used to target the erythroid-specific enhancer region of BCL11A, the editing efficiency of $CD34^+$ cells from healthy donors can reach 80%±6%. After these cells differentiate into red blood cells, the level of HbF increases from 10.5% to 29.0%. The core mechanism behind this result lies in the fact that BCL11A is a key repressor of HbF expression. Editing its enhancer region can specifically downregulate the expression of BCL11A in red blood cells, thereby relieving the repression of the γ-globin gene and ultimately activating the synthesis of HbF. In patients with SCD, this strategy is even more effective—it not only increases the proportion of HbF to 43.2% but also achieves a complete remission rate of up to 94% for VOC.

In terms of long-term efficacy, non-human primate experiments provide important support: the edited cells can colonize in vivo for more than 16 weeks, and the HbF level remains stable at 4.3%-28.6% [3]. Clinical research data also confirm this. During the 12-month follow-up period, 93.5% of patients who received this therapy did not experience severe VOC; their HbF levels continued to rise with a pan-cellular distribution, and they were completely free from transfusion dependence [4]. Notably, this strategy does not require direct repair of the mutated HBB gene—instead, it alleviates symptoms by regulating gene expression, which not only reduces technical difficulty but also makes safety easier to control. Meanwhile, editing the BCL11A enhancer only acts on the erythroid lineage and does not affect the normal function of BCL11A in other cells, effectively avoiding the risk of systemic toxicity.

2.2 Direct Repair of HBB Gene Mutations

Clinical studies have shown that after editing the hematopoietic stem/progenitor cells (HSPCs) of patients with SCD, the efficiency of correcting the E6V mutation in the HBB gene via homology-directed repair (HDR) technology can reach 30%-50%, and the sickling rate of red blood cells is significantly reduced accordingly [5]. The core value of this technology lies in its ability to directly restore the synthesis of normal hemoglobin (HbA), addressing SCD at the etiological level. However, HDR inherently has limited efficiency in HSPCs, which fortunately can be improved by optimizing culture conditions—for instance, culturing cells in a 5% hypoxic environment and adding stem cell factors not only increases HDR efficiency to over 50% but also maintains the stemness of HSPCs [6].

In addition to HDR, the application of base editors (such as ABE8e) provides a new approach for SCD treatment. It can avoid DNA double-strand breaks (DSB), achieving an editing efficiency of 54% in HEK293T cells with significantly reduced off-target effects [6]; even in HSPCs derived from patients, the HbF level can reach 27%-32% after base editing, and the edited cells can also colonize long-term and differentiate into normal red blood cells in immunodeficient mice [5]. It is worth noting that although the strategy of directly repairing HBB gene mutations can theoretically cure SCD completely, it has extremely high requirements for editing accuracy and efficiency and is still in the clinical trial stage [1].

To further break through the efficiency challenges, studies have found that adding HDR enhancers (such as RS-1) can improve editing efficiency without affecting the proliferation and differentiation capabilities of HSPCs [4]. Preclinical data also support this strategy: HSPCs repaired by HDR achieve a colonization rate of 40%-60% in non-hu-

man primate models and can continuously express normal HbA for more than 12 months; meanwhile, whole-genome sequencing confirms that the edited cells show no obvious clonal expansion or chromosomal abnormalities, and their safety has been initially verified.

2.3 Mimicking Hereditary Persistence of Fetal Hemoglobin (HPFH)

CRISPR-Cas9 can mimic the phenotype of HPFH by deleting the inhibitory binding sites in the promoter region of the γ-globin gene (such as the BCL11A binding region at position -115), thereby activating the expression of HbF. In HUDEP-2 cells, this editing can increase the γ-globin mRNA level by 38%±5% and reduce the red blood cell sickling rate by 60% [6]. A study on 3 SCD patients showed that the HbF proportion reached 19.0%-26.8% after editing, and this effect persisted for more than 18 months.

The large deletion strategy (such as deleting a 13.6kb region) enhances HbF expression by altering chromatin structure. In red blood cell precursors, the HbF level is higher than that of the strategy of single silencer inhibition [6]. This method does not require precise repair of mutations and can achieve therapeutic effects through epigenetic regulation, which is suitable for multiple SCD genotypes. However, large-fragment editing may increase the risk of chromosomal instability, and further optimization of editing accuracy is needed. The strategy of mimicking HPFH provides a new idea for SCD treatment, especially suitable for patients with HBB gene mutations that cannot be efficiently repaired by HDR.

3. Clinical Efficacy and Safety Evidence

3.1 Key Clinical Trial Results

A number of clinical trials have been conducted on different treatment strategies for SCD, and important results have been achieved. In the DREPAGREFFE-1 trial (NCT01340404), which compared allogeneic hematopoietic stem cell transplantation (alloSCT) with standard of care (SoC), 67 pediatric patients with Sickle Cell Anemia (SCA) and a history of abnormal cerebral blood flow velocity were enrolled. The results showed that at 1 year, the maximum time-averaged mean flow velocities (TAMMVs) in the alloSCT group were significantly lower than those in the SoC group (difference: -40.8 cm/s). At 3 years, the alloSCT group had lower maximum TAMMVs, a higher proportion of patients with normalized TAMMVs, lower stenosis scores in patients without stroke, and better quality of life in terms of physical and school function. In a 10-year follow-up study, significant differences in various physiological indicators were observed between the allogeneic hematopoietic stem cell transplantation (alloSCT) group and the SoC group. Compared with the SoC group, patients in the alloSCT group had higher hemoglobin (Hb) levels and normal hemoglobin (HbA) percentage, while the sickle hemoglobin (HbS) percentage, reticulocyte count, white blood cell (WBC) count, neutrophil count, bilirubin level, lactate dehydrogenase (LDH) level, and ferritin level were all lower. This difference was statistically significant (P<.001). Beyond physiological indicators, the differences between the two groups in MRI/MRA imaging findings and cognitive performance also merit attention. At the initial enrollment of the study, 6 out of 31 patients in the SoC group had silent cerebral infarction (SCI), and 12 out of 28 patients in the alloSCT group had SCI. However, by the 10th year of follow-up, 5 additional patients in the SoC group developed SCI, whereas no new SCI cases occurred in the alloSCT group. This further demonstrates the advantage of alloSCT in providing long-term protection for patients‘ nervous systems. Moreover, SCI was no longer visible or became <3 mm in 1 patient (SoC group) and 4 patients (alloSCT group) (P=.010) [4].

A researcher-initiated trial (IIT) of the base-editing drug CS-101 Injection for SCD conducted by Zhengxu Biotechnology successfully cured the first patient. After treatment, the patient‘s HbF level increased significantly and continuously, while the sickle hemoglobin (HbS) level decreased significantly and continuously. From 6 months after treatment, the ratio of HbF to HbS was stably maintained at 6.5:3.5, and the total hemoglobin concentration was stabilized above 120 g/L. No vaso-occlusive crisis (VOCs) occurred within 6 months after treatment, and the patient has returned to normal life. After receiving tBE-edited autologous stem cell transplantation in February 2025, the patient achieved neutrophil engraftment on day 13, and the platelet concentration reached above 50×10^9/L on day 21. One month after treatment, the fetal hemoglobin proportion increased rapidly from the pre-treatment baseline of 4.4% to 34.6%. Three months after treatment, the fetal hemoglobin proportion remained above 60%, the sickle hemoglobin proportion remained below 40%, and no product-related adverse events were observed [6].

These clinical trial results indicate that alloSCT has significant advantages in improving the physical indicators, quality of life, and cognitive function of SCD patients; while the base-editing drug CS-101 Injection shows good efficacy in increasing the patient‘s hemoglobin level and eliminating vaso-occlusive crisis, bringing new hope and options for SCD treatment.

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3.2 Safety Characteristics

In terms of off-target effects, although no off-target genome editing has been observed in the evaluation of edited CD34+ cells from healthy donors and patients, the possibility of unexpected off-target editing in individual CD34+ cells cannot be completely excluded due to individual genetic variations, and its potential clinical impact remains unclear. Deep sequencing of more than 5,000 potential off-target sites showed no obvious off-target editing, and the application of high-fidelity Cas9 variants further reduces this risk [7].

The risks related to preconditioning mainly focus on myeloablative regimens. Taking busulfan as an example, as a commonly used myeloablative drug, it belongs to bifunctional alkylating agents of the dimethyl sulfonate class. In clinical application, approximately 15% of patients who received standard-dose busulfan preconditioning developed mild to moderate hepatotoxicity, and 1 case progressed to grade 3 veno-occlusive disease (VOD), which was relieved after treatment with defibrotide [8]. To reduce the toxicity during the preconditioning stage, low-intensity preconditioning regimens have been developed, such as low-dose busulfan combined with immunosuppressants. Clinical trials have shown that the incidence of toxic reactions of this regimen is reduced by approximately 40% compared with the standard regimen, while the colonization efficiency of edited cells is not affected.

In addition to off-target effects and preconditioning-related risks, the safety of CRISPR-Cas9 therapy also involves long-term genomic stability. In the long-term follow-up of patients receiving Casgevy treatment, no clonal dominant expansion of edited cells was found, which is consistent with the results of non-human primate experiments—no abnormal proliferation of edited HSPCs was observed during the 16-week follow-up [9]. Deep sequencing of more than 5,000 potential off-target sites showed no obvious off-target editing, and the use of high-fidelity Cas9 variants (modified at specific amino acid sites of the Cas9 protein to change its binding properties with DNA and improve specificity) further reduces this risk.

In addition, the electroporation technology used in the CRISPR-Cas9 editing process may cause certain damage to HSPCs, resulting in a cell viability decrease of approximately 5%-10%. Studies have shown that optimizing electroporation parameters—such as appropriately reducing voltage and shortening pulse duration—not only controls the loss of cell viability within 5% but also maintains an editing efficiency of over 80%, effectively balancing efficiency and cell damage during the CRISPR-Cas9 editing process [7].

In conclusion, under the current technical system, the safety of CRISPR-Cas9 therapy can be effectively managed. Mature response strategies have been developed for various safety concerns, ranging from the prevention and control of off-target effects, the reduction of preconditioning toxicity, to the control of cell damage. However, it should be noted that there is still insufficient data to support the long-term genomic stability of this technology beyond 10 years. In the future, more long-term follow-up observations and in-depth studies are needed to further verify its long-term safety.

4. Existing Challenges and Solutions

4.1 Technical Challenges

A key challenge in the clinical translation of CRISPR-Cas9 lies in the stability of its editing efficiency. Particularly in HSPCs, the efficiency of HDR-mediated gene repair is often less than 30%, which directly results in insufficient HbF levels (below 20%) in some patients after treatment, failing to achieve the desired therapeutic effect [6]. To address this issue, researchers have explored effective solutions: by adding HDR enhancers (such as RS-1) to the editing system while optimizing cell culture conditions (e.g., using a 5% hypoxic environment), not only can the HDR efficiency in HSPCs be increased to over 50%, but the stemness of the cells can also be preserved to the greatest extent, providing a guarantee for subsequent in vivo engraftment and the exertion of hematopoietic function. In addition, combined targeting of multiple HbF repressor genes (such as simultaneous editing of BCL11A and ZBTB7A) can synergistically increase HbF expression, with an efficiency increase of approximately 30% compared with single-target editing [7].

The optimization of delivery systems is also crucial. The currently commonly used electroporation technology can achieve high editing efficiency, but may cause 5%-10% cell death. The new lipid nanoparticle (LNP) delivery system can increase the editing efficiency by 2-3 times with significantly reduced cytotoxicity. In preclinical studies, LNP-mediated CRISPR component delivery maintained the viability of HSPCs above 90%. Viral vectors (such as rAAV6) show advantages in delivering donor templates and can improve HDR efficiency, but there is a risk of immunogenicity, with approximately 8% of patients developing anti-AAV antibody responses.

4.2 Clinical Implementation Barriers

The high treatment cost is a major obstacle to the popularization of CRISPR-Cas9 therapy. The single-treatment cost of Casgevy is approximately 2.2 million US dollars,

far exceeding that of traditional HSCT (100,000-200,000 US dollars). The main reasons for the high cost include personalized cell preparation, strict quality control, and long-term follow-up requirements. To reduce costs, the application of automated cell processing platforms can reduce the preparation cost by approximately 40% while improving batch-to-batch consistency. In addition, the development of universal edited cells (such as allogeneic HSPC editing) is expected to further reduce costs, and they have shown therapeutic effects comparable to autologous edited cells in mouse models.

The limitation of applicable populations also needs to be addressed urgently. Currently, Casgevy is only approved for patients over 12 years old with frequent VOC, while the therapeutic benefits for children and asymptomatic patients are not yet clear [5]. In small-scale clinical trials involving children aged 5-11 years, CRISPR-Cas9 therapy showed similar safety and efficacy to that in adults, with HbF levels reaching above 35% and no serious adverse reactions. This provides a basis for expanding the applicable population, but large-scale clinical trials are still needed for verification [10].

4.3 Long-term Efficacy and Efficacy Stability

Some patients experience a decrease in HbF levels by approximately 10%-15% within 1-2 years after treatment, which may be related to insufficient proliferative advantage of edited cells. Studies have found that editing the HOXB4 gene (which regulates stem cell self-renewal) can enhance the long-term colonization ability of edited cells, maintaining the HbF level stable during 2-year follow-up (decrease <5%) [6]. In addition, the combined use of hydroxyurea and CRISPR-Cas9 therapy can rapidly increase HbF levels through drugs, making up for the insufficient initial proliferation of edited cells. In clinical trials, this combined regimen increased the patient‘s HbF level by approximately 15% compared with single therapy [3].

Long-term efficacy is also related to the differentiation potential of edited cells. Studies have shown that if the edited HSPCs maintain a high CD34+CD38- ratio (>20%) during in vitro culture, they have stronger long-term in vivo colonization ability and more stable HbF levels. Therefore, optimizing cell sorting and culture processes to retain more HSPCs with long-term hematopoietic potential is the key to maintaining long-term efficacy [9]. These strategies provide new directions for maintaining long-term efficacy, but large-scale clinical trials are still needed for verification.

5. Conclusion

This article systematically reviews the application of CRISPR-Cas9 gene editing technology in the treatment of SCD, covering core strategies such as targeting the BCL11A gene enhancer to activate HbF, directly repairing HBB gene mutations, and mimicking HPFH. Combined with clinical trial data, it confirms that this technology can significantly increase HbF levels, reduce the incidence of VOC, and ensure controllable short-term safety. The aforementioned achievements provide a potential curative solution for SCD, a globally prevalent monogenic inherited disease. In particular, they overcome the limitations of traditional treatments—such as the need for lifelong administration of hydroxyurea and the scarcity of donors for HSCT—responding to the urgent demand for innovative therapies mentioned in the introduction and offering important references for the translational application of gene editing technology in the treatment of monogenic diseases.

However, the field still faces limitations, including unclear long-term genomic stability and high treatment costs. Additionally, this article does not analyze the differences in therapeutic efficacy among patients with different genotypes. Future research should focus on technical optimization (e.g., improving editing efficiency and the safety of delivery systems), cost control (e.g., automated cell preparation), and expanding the applicable population. Meanwhile, large-scale long-term follow-up studies should be conducted to promote the transition of CRISPR-Cas9 therapy from the laboratory to widespread clinical application, bringing hope for a cure to SCD patients worldwide.

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