CRISPR-Cas9 Technology in the Treatment of Sickle Cell Anemia (SCA)

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Abstract. Sickle cell anemia (SCA) is a monogenic inherited disease caused by a single point mutation in the HBB gene. Traditional treatments are ineffective in fundamentally correcting the genetic defect. In recent years, CRISPR-Cas9, an emerging gene editing technology, has shown great potential in the treatment of SCA. This article systematically reviews the mechanism of action of CRISPR-Cas9 and compares it with other editing tools. We focus on its application in SCA treatment, including strategies such as repairing the HBB gene mutation or targeting the HBG1/2 promoter to activate fetal hemoglobin. Furthermore, based on data from cell-based experiments, animal models, and clinical trials, we discuss the efficacy and limitations of representative therapies such as CTX001, Casgevy, and Lyfgenia. Results have shown that CRISPR-Cas9 can significantly improve red blood cell function in patients, and some therapies have received clinical approval, marking a significant breakthrough in its transition from laboratory research to clinical application. However, challenges remain, such as off-target effects, insufficient delivery efficiency, high treatment costs, and transplant-related complications, which need to be addressed. In the future, emerging strategies such as RNA editing are expected to expand into broader areas, including tumor treatment, while improving safety, providing safer and more accessible treatment options for monogenic diseases such as SCA.

Keywords: CRISPR-Cas9, sickle cell anemia, β-globin gene.

1. Introduction

In the wave of precision medicine development, gene editing technology, with its ability to rewrite genetic codes, is reshaping the traditional paradigm of disease treatment. Among them, the CRISPR-Cas9 system has evolved from a bacterial immune mechanism into a "genetic scalpel" for human disease treatment. Its characteristics of precise targeting, flexible design, and controllable cost bring the possibility of curing intractable diseases such as monogenic genetic disorders, promoting the transformation of medical models from "symptom relief" to "etiology correction" [1, 2].

Sickle Cell Anemia (SCA) is a globally prevalent monogenic genetic disorder. Its pathogenic root lies in a point mutation (GAG→GTG) in the β-globin gene (HBB), which leads to abnormal polymerization of hemoglobin S (HbS), triggering red blood cell sickling, hemolysis, and a series of symptoms such as pain crises and organ failure [3,4]. The global number of patients has exceeded 7 million, with African descendants accounting for 60%. Traditional treatment methods face multiple dilemmas in addressing this disease: drug therapy can only relieve symptoms, and long-term use is prone to cause myelosuppression, failing to fundamentally correct genetic defects [5]; blood transfusion therapy relies on blood supply and is likely to induce iron overload, infections, and other issues, making it difficult to prevent disease progression [4]; hematopoietic stem cell transplantation is limited by donor matching—only 10% of patients can find a fully HLA-matched sibling donor, and the risk of post-transplant graft-versus-host disease (GVHD) is as high as 30% [2].

The precise nature of CRISPR-Cas9 is highly compatible with the monogenic pathogenic mechanism of SCA. By repairing HBB gene mutations or activating fetal hemoglobin (HbF) expression, it is expected to fundamentally correct the pathological process [6,4]. As of 2024, related therapies such as CTX001 have entered Phase III clinical trials, initially verifying the therapeutic potential of this technology [5,7]. In addition, a study that targeted disruption of the promoters of HBG1 and HBG2 (γ-globin) genes using CRISPR-Cas9 technology can significantly increase fetal hemoglobin levels in patients' red blood cells, providing a new strategy for disease treatment [8].

More milestone-worthy is that from the end of 2023 to the beginning of 2024, regulatory authorities in many countries around the world successively approved CRISPR-Cas9 gene editing therapies for the treatment of SCA, marking the official transition of this technology from clinical research to clinical application [9]. The clinical data and regulatory history of these two approved therapies (Casgevy and Lyfgenia) provide key practical evidence for gene editing in the treatment of monogenic genetic disorders and serve as an important milestone in the clinical translation of CRISPR-Cas9 technology. However, issues such as off-target risks, delivery efficiency, and cost barriers still restrict its widespread application, requiring further in-depth analysis of relevant mechanisms and optimization strategies [5, 7].

This article will systematically sort out the technical principles of CRISPR-Cas9 and compare the characteristics of different gene editing tools; focus on the treatment strategies for SCA, and deeply analyze the efficacy and limitations of existing therapies by combining in vitro experiments, animal models, and clinical trial data (e.g., HBB repair experiments [6], CTX001 trials [4], HBG1/2 promoter editing studies [8], and clinical data of approved therapies [9]); finally, provide theoretical references for technical optimization and clinical translation to facilitate the breakthrough of "curative therapies" for SCA.

2. Principles of CRISPR-Cas9 Technology

CRISPR-Cas9 originates from the adaptive immune system of bacteria. Its natural CRISPR locus consists of repetitive sequences and spacer sequences, where the spacer sequences can record DNA fragments of phage invasion; Cas9, as an endonuclease, contains two domains (HNH and RuvC), which are responsible for cutting the complementary strand and non-complementary strand of DNA, respectively [1]. After artificial modification, the guide RNA (gRNA) mimics the function of spacer sequences to guide Cas9 in recognizing and cutting target DNA, thereby achieving gene editing.

The working process of CRISPR-Cas9 mainly includes three steps: first, targeted recognition—the 20nt sequence of gRNA is complementary to the target DNA, and Cas9 recognizes the PAM sequence (NGG) to lock the cleavage site [1]; second, induction of double-strand breaks (DSB)—the HNH and RuvC domains of Cas9 work together to cut the double strands of DNA, activating the cell's repair mechanism; third, repair selection—cells can undergo rapid repair through Non-Homologous End Joining (NHEJ), a process that easily introduces base insertions or deletions to achieve gene knockout (e.g., knockout of BCL11A [4]); alternatively, they can achieve gene correction through Homology-Directed Repair (HDR), which enables precise gene replacement or insertion with the participation of external templates (e.g., repair of HBB mutations [6]).

In the field of genetic disease treatment, CRISPR-Cas9 shows significant advantages compared with other gene editing tools such as ZFN and TALEN [3]. In terms of targeting efficiency, CRISPR-Cas9 can reach 92%, higher than 78% of ZFN and 85% of TALEN; in terms of in vitro off-target rate, CRISPR-Cas9 is 0.05%, lower than 0.12% of ZFN and 0.08% of TALEN; in terms of design cost, CRISPR-Cas9 is approximately 500 US dollars, far lower than 5,000 US dollars of ZFN and 3,000 US dollars of TALEN; the design cycle of CRISPR-Cas9 is only 1–2 weeks, shorter than 4–6 weeks of ZFN and 3–4 weeks of TALEN. ZFN and TALEN require customized proteins, resulting in complex design and high costs, while CRISPR only needs to synthesize gRNA. Its flexibility and economy are more suitable for clinical translation needs, which also lays a technical foundation for the subsequent approval of therapies such as Casgevy and Lyfgenia [9].

3. CRISPR-Cas9-Based Treatment Strategies for SCA

3.1. Repair of HBB Gene Mutations

A treatment strategy targeting HBB gene mutations in SCA involves HDR-mediated repair by CRISPR-Cas9, which replaces the mutant GTG sequence with the normal GAG sequence to restore the expression of HbA and fundamentally correct the pathological process [6]. In an in vitro stem cell

repair experiment [6], researchers isolated CD34+ hematopoietic stem cells from patients, used AAV6 vectors to deliver HDR templates and gRNA-Cas9 complexes, and optimized the multiplicity of infection (MOI) to 50. The results showed that 22% of CD34+ cells achieved precise repair; the proportion of HbA in differentiated red blood cells reached 25%, and the sickling rate decreased by 40%; whole-genome sequencing only identified 3 non-target sites, with signal intensity lower than 0.1%, indicating a low off-target risk. However, this strategy also has limitations: most hematopoietic stem cells are in the G0 resting phase, and HDR relies on cell division, leading to limited repair efficiency; meanwhile, although no integration was observed in the experiment with AAV vectors, long-term safety still needs further verification.

3.2. Two Core Strategies for Activating HbF Expression

3.2.1. Knockout of BCL11A Enhancer

HbF ($\alpha_2\gamma_2$) can inhibit the polymerization of HbS, but its expression decreases sharply after birth due to the inhibitory effect of the BCL11A gene. Knocking out the enhancer region of BCL11A using CRISPR-Cas9 can relieve the inhibition of the γ-globin gene, increase HbF levels, and compensate for the function of HbS [1,4]. The representative therapy of this strategy is Casgevy (exagamglogene autotemcel), whose clinical data have supported regulatory approvals in multiple countries [9].

In the single-arm multicenter study supporting the approval of Casgevy [9], enrolled SCA patients were required to meet the condition of "at least 2 severe vaso-occlusive events per year in the two years before screening". The treatment process was similar to that of CTX001: autologous CD34+ hematopoietic stem cells were collected from patients, edited at the BCL11A enhancer by CRISPR-Cas9, and then infused back after myeloablative conditioning (e.g., busulfan). Efficacy data showed that among 31 patients with sufficient follow-up time, 29 (93.5%) achieved the primary endpoint of "no severe vaso-occlusive events for at least 12 months within 24 months of follow-up"; all patients successfully achieved cell engraftment, with no cases of graft rejection or failure. In terms of safety, common adverse events included thrombocytopenia, leukopenia, nausea, oral ulcers, musculoskeletal and abdominal pain, and febrile neutropenia, with no severe fatal adverse reactions [9].

Based on the above data, on November 16, 2023, the UK MHRA took the lead in approving Casgevy for SCA patients aged ≥12 years with recurrent vaso-occlusive crises and no HLA-matched related donors [9]; on December 8, 2023, the US FDA approved it for the same indication [9]; on December 15, 2023, the EU EMA also approved this therapy for the treatment of SCA [9], making it one of the world's first CRISPR-Cas9 therapies approved for this disease [9]; on January 16, 2024, the US FDA further approved the Biologics License Application (BLA) for Casgevy, expanding its indication to transfusion-dependent β-thalassemia [9].

In the previous CTX001 clinical trial, two patients were a 26-year-old transfusion-dependent patient (transfused once a month, with 8 pain crises per year) and a 19-year-old patient (12 pain crises per year, with impaired organ function) [7]. The treatment process involved collecting autologous CD34+ stem cells, editing the BCL11A enhancer with CRISPR-Cas9, and infusing the edited cells back after myeloablative conditioning (busulfan + fludarabine, AUC=10,000 μmol·min). Efficacy data showed that the first patient had an HbF proportion of 48% 6 months after transplantation, stopped blood transfusion, and the effect persisted for 12 months of follow-up; the second patient had an HbF proportion of 52%, and the number of pain crises dropped to 0, with a significant improvement in quality of life. In terms of safety, grade 3–4 neutropenia (lasting 14 days) and thrombocytopenia (lasting 21 days) occurred in the short term, with no septic shock or hepatic veno-occlusive disease (VOD); during 3 years of long-term follow-up, 5% of patients developed mild clonal hematopoiesis, but it did not progress to myelodysplastic syndrome (MDS) [5]. Currently, this strategy has entered the clinical approval stage, with CTX001 receiving accelerated approval from the FDA [7]. It has clear efficacy and is suitable for most patients, but long-term clonal hematopoiesis risks need to be vigilant, and further improvement can be achieved by optimizing editing targets (e.g., more precise enhancer regions).

3.2.2. Editing of HBG1 and HBG2 Promoters

In a multicenter Phase I-II clinical trial conducted by Akshay Sharma's team in 2023 [8], a CRISPR-Cas9-edited CD34+ hematopoietic stem and progenitor cell product (OTQ923) was developed to activate HbF expression through a novel mechanism. Different from knocking out the BCL11A enhancer, this strategy directly targets the transcriptional repressor elements in the promoter regions of HBG1 and HBG2 genes, using gRNA-68 to directionally disrupt the repressor sites (targeting 246bp upstream of the transcription start site of the two genes), thereby relieving the transcriptional inhibition of the γ-globin gene and further increasing HbF levels [8].

A recent study evaluated the efficacy and safety of CRISPR-Cas9-based therapy OTQ923 for Sickle Cell Disease (SCD). In preclinical experiments, CRISPR-Cas9 tiling screening on CD34+ cells from healthy donors identified gRNA-68 as the most effective, inducing the highest proportion of fetal hemoglobin-immunostained red blood cells (F cells). Editing efficiency in donor-derived CD34+ cells reached 80.5±9.8%, with 60.5±6.8% F cells after differentiation (vs. 22.9±3.5% in controls). Similar results were achieved in CD34+ cells from SCD patients, with editing efficiency of 85.8±14.7% and 65.4±12.1% F cells (vs. 39.6±12.4% in controls). SITE-Seq identified 279 potential off-target sites, but no mutations were detected, and transplantation into immunodeficient mice confirmed long-term engraftment and preserved stemness. In the clinical phase, three young adults with severe SCD (βS/βS genotype) received autologous OTQ923 after plerixafor-mobilized CD34+ cell collection, CRISPR-Cas9 editing, and busulfan myeloablation. During 6–18 months of follow-up, all patients achieved stable engraftment (neutrophils: 18–26 days; platelets: 29–44 days), with fetal hemoglobin levels rising to 19.0%–26.8% and F cells to 69.7%–87.8%. SCD-related symptoms markedly improved, with adverse events attributed only to busulfan or underlying disease. At one year, bone marrow showed restored balanced trilineage hematopoiesis without dysplasia, supporting the therapeutic potential of OTQ923.

The advantage of this strategy lies in more precise targeting—only disrupting the repressor elements in the HBG1/2 promoters, avoiding potential impacts on erythropoiesis that may be caused by complete knockout of BCL11A [8]; however, limitations also exist: the currently induced HbF level is still insufficient to completely inhibit HbS polymerization, and all 3 patients had mild persistent hemolysis (manifested by increased reticulocyte count). In the future, the efficacy needs to be further improved by optimizing editing efficiency (e.g., increasing the targeted editing frequency of long-term repopulating hematopoietic stem cells) [8].

3.2.3. Newly Approved Therapy

In addition to the above strategies, the FDA also approved another CRISPR-Cas9 gene editing therapy, Lyfgenia (lovotibeglogene autotemcel), for the treatment of SCA [9]. Different from "activating HbF" or "repairing HBB", its mechanism involves modifying patients' hematopoietic stem cells through lentiviral vectors to express HbAT87Q—a mutant hemoglobin with functions similar to normal adult hemoglobin (HbA), which can reduce red blood cell sickling and improve vascular blood flow [9].

In the single-arm multicenter study supporting the approval of Lyfgenia, SCA patients aged 12–50 years with a history of vaso-occlusion received treatment. The efficacy evaluation indicator was "complete remission of vaso-occlusive events 6–18 months after infusion", and the results showed that 88% (28/32) of patients achieved this endpoint. In terms of safety, common adverse events included stomatitis, thrombocytopenia, leukopenia, and febrile neutropenia; however, it is important to note that cases of hematological malignancies have been reported in patients receiving this therapy. Therefore, the FDA added a black box warning for it, recommending long-term follow-up of treated patients to monitor this risk [9].

3.2.4. Emerging Strategies

Emerging strategies include base editing and multigene editing [7]. Base editing does not require inducing double-strand breaks and can directly mutate the GTG sequence of HBB to GAG, reducing the off-target rate by 50% compared with traditional CRISPR; multigene editing simultaneously edits

BCL11A (to increase HbF) and HBB (to repair mutations), constructing a "dual guarantee" to enhance efficacy. In preclinical studies, the base editor BE4 achieved an 80% single-base correction efficiency in in vitro cell experiments, increasing the proportion of HbA to 35% [7]; in mouse models, combined editing increased the proportion of HbF+HbA to 70% and reduced the red blood cell sickling rate by 90%, but the complexity of the delivery system doubled due to the need to introduce two types of gRNA simultaneously. These emerging strategies overcome the limitations of traditional double-strand breaks, but their technical maturity is low and delivery is difficult, requiring more preclinical verification. They are expected to become more precise treatment options in the future.

3.2.5. 2023 NEJM New Clinical Study

A multicenter, open-label Phase II extension trial evaluated the long-term efficacy and safety of CRISPR-Cas9-edited autologous hematopoietic stem cell therapy in 45 patients with SCA aged 12–65 years, including 12 with the HbSC subtype, a population rarely studied previously. Eligible patients had at least two vaso-occlusive crises per year or required regular transfusions and lacked HLA-matched donors. The protocol used autologous CD34+ cell editing and myeloablative infusion, with optimized mobilization by plerixafor plus G-CSF, which increased CD34+ yield by 20% and reduced apheresis procedures. With a median follow-up of 24 months, 43 patients (95.6%) remained free of severe vaso-occlusive crises for 18 months. In HbSC patients, sickled red blood cells decreased by 35% (vs. 28% in βS/βS), and 92% of transfusion-dependent patients achieved transfusion independence, except one with <30% editing efficiency. Edited cells showed durable engraftment, with 45%–62% persisting in bone marrow and stable HbF expression (22%–31% of total hemoglobin) after 2 years. Safety outcomes were favorable: individualized busulfan dosing (AUC 75–90 mg·hour/L) shortened grade 3–4 neutropenia to 10–12 days, with no hepatic veno-occlusive disease. Among 1,000 off-target sites screened, only one low-frequency (<0.02%) event was detected, with no clinical impact, confirming both efficacy and safety of the therapy [10].

4. Conclusion

Currently, although CRISPR-Cas9 technology has achieved breakthroughs in clinical approval for the treatment of SCA, it still faces many technical bottlenecks and clinical challenges. Although off-target risks have been reduced through high-fidelity Cas9 and optimized gRNA, they are still difficult to completely avoid in complex genomic environments; in terms of delivery efficiency, viral vectors (e.g., lentivirus, AAV) have immunogenicity issues, while non-viral vectors (e.g., lipid nanoparticles) have low efficiency; cost barriers are significant—for example, the cost of a single treatment with CTX001 exceeds 2.2 million US dollars, and the newly approved Casgevy and Lyfgenia, as similar cell therapies, are expected to maintain high costs, limiting their popularization worldwide (especially in low- and middle-income regions with a high incidence of SCA). In addition, issues such as mild hemolysis caused by insufficient fetal hemoglobin levels in the HBG1/2 promoter editing strategy, potential toxicity of myeloablative conditioning drugs (e.g., busulfan), and the risk of hematological malignancies indicated by Lyfgenia all need to be further addressed in subsequent studies.

It is worth noting that to address the safety concerns of DNA editing, RNA editing therapy has become a new research focus—RNA editing therapies for α-1 antitrypsin deficiency (AATD) and Stargardt disease (a type of juvenile macular dystrophy) were approved for clinical research. Different from CRISPR-Cas9, RNA editing does not alter the genome; it only corrects RNA sequences through single-base editing (e.g., using ADAR enzymes to replace adenine with inosine in RNA) or exon editing, thereby producing functionally normal proteins with higher safety. In addition, RNA editing can be extended to the field of malignant tumor treatment by generating proteins that inhibit tumor cell growth or replacing RNA sequences related to tumor growth factors, and is expected to provide safer alternative treatment options for monogenic genetic disorders such as SCA in the future.

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