Proceedings of the 5th International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/2027.39064

CRISPR Gene Editing Therapy for Sickle Cell Disease and Beta-Thalassemia: Centered on the Fetal Hemoglobin Reactivation Pathway

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Abstract. β-thalassemia and sickle -cell disease are both serious inherited blood disorders caused by hemoglobin subunit beta (HBB) gene mutations. While conventional treatments such as blood transfusion combined with iron chelation therapy and allogeneic stem cell transplantation, they often require long-term medical support and many carry risks, especially in younger patients. Meanwhile, recent advances in CRISPR-Cas9 gene editing have made it possible to explore more precise genetic therapies, although the clinical use of this technology is still limited by safety concerns and the need for more reliable delivery methods. This study aims to investigate the clinical application potential of CRISPR-series editing technologies, with HbF reactivation pathways as the central focus. Research findings indicate that the BCL11A enhancer target within the CRISPR-Cas9 system demonstrates greater clinical translation maturity; however, when using base editing to target the HBG promoter, this approach outperforms CRISPR-Cas9-mediated BCL11A targeting across multiple key aspects, including HbF enhancer editing strategies and hematopoietic reconstitution. We also found that SCD patients struggle to mobilize their hematopoietic stem cell. Furthermore, this paper systematically summarizes the complete workflow of this gene editing therapies and defines the current limitations and technical gaps in clinical translation. The clinical shortcomings and mechanistic controversies synthesized herein may serve as a literature basis and provide research directions for the future development of low-toxicity conditioning regimens, novel hematopoietic mobilization agents, and precise fetal hemoglobin efficacy assessment systems.

Keywords: CRISPR-Cas9 gene editing, Sickle Cell Disease, β-thalassemia, Fetal hemoglobin

1. Introduction

Sickle cell disease (SCD) and transfusion-dependent beta-thalassemia (TDT) are two highly prevalent inherited hemoglobinopathies, both of which are induced by mutations in the HBB (hemoglobin subunit beta) gene. SCD arises from a single-base mutation that generates abnormal HbS, leading to erythrocyte sickling and vascular occlusion under hypoxic conditions; TDT, in contrast, results from hundreds of distinct mutations causing β-globin deficiency, which in turn

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induces ineffective erythropoiesis and chronic hemolysis [1]. Conventional therapies provide only symptomatic relief, while allogeneic hematopoietic stem cell transplantation is limited by donor shortage, graft-versus-host disease (GVHD), and iron overload, and these limited its clinical application [2]. CRISPR-Cas9 is originally an adaptive immune system employed by bacteria (archaea) to defend against bacteriophage invasion. This effector protein possesses nuclease activity, enabling it to induce DNA double-strand breaks (DSBs) in hematopoietic stem cells and, through insertion/deletion (indel) mutations, provide a potential approach for autologous curative therapy. Currently, two mainstream editing pathways are the BCL11A erythroid-specific enhancer and the HBG1/HBG2 promoter. The former has completed large-scale phase III clinical trials and received marketing approval, while the latter one has also demonstrated favorable therapeutic efficacy in early-stage clinical trials.

However, although both routes drive fetal hemoglobin induction, the two pathways act through entirely separate molecular circuits. This divergence leaves a critical gap in the available evidence: a dedicated synthesis comparing their mechanistic foundations and safety profiles is still absent. A further limitation concerns patient recruitment—cohorts from middle-aged and elderly populations are heavily underrepresented in completed trials. Consequently, the molecular basis for age-dependent variation in therapeutic potency remains development and the translational risks inherent to each editing modality have yet to be comprehensively assessed. Against this backdrop, the present work focus on HbF reactivation. It summarizes clinical findings for CRISPR-Cas9, base editing, and prime editing in hematopoietic stem cell engineering for SCD and TDT, cross-compares therapeutic performance and safety across distinct genomic targets, and unpacks the full scope of procedural risks and translational bottlenecks. This review ultimately establishes an evidence base to guide the future refinement of customized gene-therapy regimens.

2. Reactivation of CRISPR editing targets by HBF and its mechanism of action

2.1. CRISPR-cas9-mediated modification of the GATA-1 binding site within the BCL11A gene

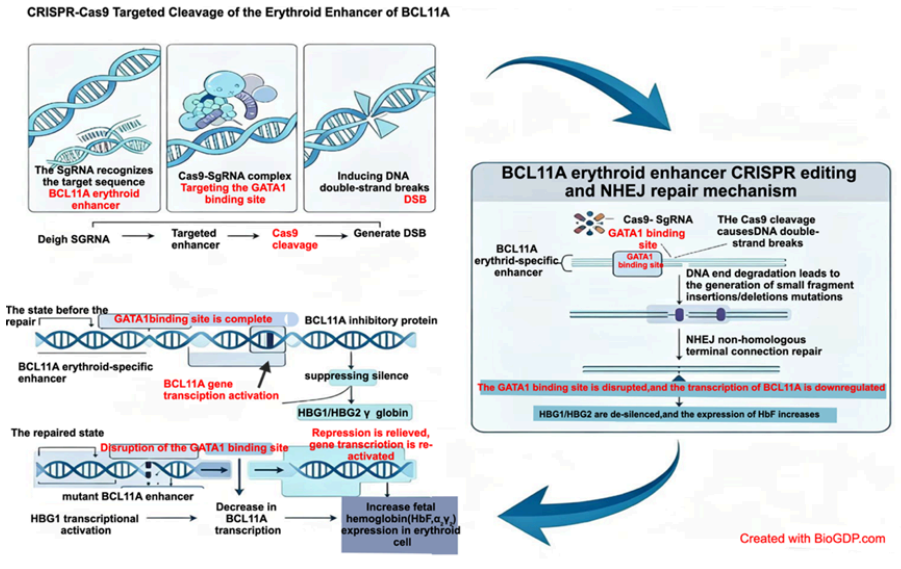


Figure 1. HBG1/2 promoters and BCL11A erythroid enhancer as genomic targets to reactivate fetal hemoglobin (HbF) expression for the treatment of beta-hemoglobinopathies

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This pathway mechanism involves targeted knockout of the BCL11A erythroid-specific enhancer, inducing DNA double-strand breaks via CRISPR to fundamentally ameliorate hemolysis and vasculopathy (Figure 1) [3]:

2.2. HBG1/HBG2 promoter modification (in situ regulation of downstream target genes)

There are some mechanistic differences between HBG1/HBG2 and BCL11A targeting. Targeting the HBG1/HBG2 promoter eliminates local BCL11A binding motifs via site-specific DNA cleavage, which derepresses γ-globin transcription without altering overall cellular BCL11A expression [4]. In contrast, editing the BCL11A erythroid enhancer activity, leading to global downregulation of BCL11A transcription and protein abundance. Both strategies restore fetal hemoglobin expression through distinct upstream regulatory mechanisms (Figure 2).

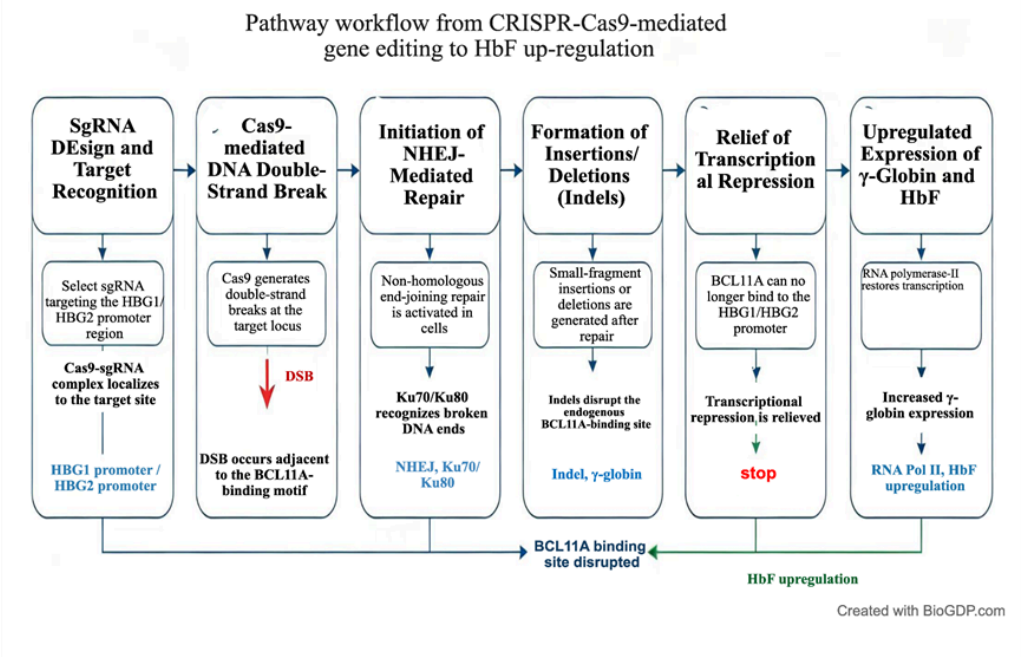


Figure 2. Mechanism of CRISPR-Cas9-mediated cleavage of the HBG1/HBG2 promoter

2.3. A multidimensional comparison of CRISPR-cas9 gene editing strategies targeting the BCL11A enhancer and the HBG1/HBG2 promoters

To facilitate a direct comparison of the technical, clinical, and safety profiles of two mainstream CRISPR-Cas9 HbF reactivation editing targets, multicenter clinical trial and publicly available regulatory review data have been compiled as presented in the table1.

Table 1. Multidimensional comparison of gene editing strategies targeting the BCL11A enhancer and the HBG1 HBG2 promoter

The dimension of comparison	Editing of the GATA-1 site within the BCL11A enhancer	Silencer-site editing of the HBG1/HBG2 promoter	
In-vitro editing efficiency in CD34⁺ cells (Indel-positive rate)	82.6%~86.0%±2.5%	88.5% ±3.1%, slightly higher than that of the BCL11A target	[4]

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Table 1. (continued)

Off-target mutation frequency (whole-genome screening via GUIDE-seq)	Extremely low, no significant pathogenic off - targets; only extremely low - frequency intergenic mismatched cleavage, no coding-regions off - target.	One candidate off-target site in non-coding region; detected for the HBG1-HBG2 target under parallel experimental conditions.	[5, 6]
Steady-state HbF induction level (clinical mean value)	HbF accounts for 39.6%-49.6% of total hemoglobin.	HbF: 30%-44% (the β-thalassemia major cohort); 19%-26.8% (SCD cohort at steady-state)	[5, 6]
Clinical maturity / regulatory approval status	FDA- approved 2023 (Casgevy / Exa-cel). Phase-III clinical trial completed, over 45 months follow-up.	Only phase-I/II clinical trials completed; no commercial product available; follow-up ≤ 24 months.	[6, 7]
Eligible patient population	All-age patients (SCD) or transfusion-dependent β-thalassemia (TDT) (for patients aged ≥12 years; pediatric expansion trial ongoing).	Priority for β^0/β^0 severe β-thalassemia; limited sample size for SCD clinical cohorts.	[5, 6]
Long-term safety risks	BCL11A is downregulation restricted to erythroid-lineage cells. Hematopoietic stem cells and lymphocytes retain intact BCL11A function; no clonal hematopoiesis or immune developmental abnormalities have been observed.	Direct globin gene modification.; rare 4.9-kb large-fragment deletion between HBG1-HBG2 may occur; long-term chromosomal stability remains to be validated.	[6, 8]
Conditioning regimen / differences in hematopoietic reconstitution	TDT: neutrophil engraftment: 29 days, platelet engraftment: 43 days.SCD: neutrophil engraftment: 27 days, platelet engraftment: 32 days.	No publicly available human clinical-trial data on hematopoietic engraftment have been reported for this target to date	

Footnote: The two gene editing strategies were evaluated in independent clinical trials. No prospective head to head comparative trial is available. Since data were obtained from separate study cohorts, efficacy and safety cannot be directly compared on an equivalent basis.

From an overall perspective, the BCL11A target exhibits greater stability and feasibility under CRISPR-Cas9 technology, whereas HBG1/HBG2 promoter editing only cis-disrupts the inhibitory effect on γ-globin from a single chromosome, thereby imposing an upper limit on HbF expression. Moreover, this gene cluster region is densely populated with homologous repetitive sequences, rendering it prone to aberrant recombination during repair following Cas9 cleavage. Consequently, the steady-state HbF levels achieved after editing are typically lower than those resulting from BCL11A enhancer editing, and this locus is more susceptible to large genomic deletions. However, single-base editing targeting the HBG1/HBG2 locus achieves HbF induction levels (accounting for 55%–64% of hemoglobin [9]), off-target mutation frequency (the risk of single-nick mispairing is substantially lower than that of Cas9 double-strand cleavage) and pre-conditioning/hematopoietic reconstitution differences confer greater advantages.

2.4. Emerging derivative gene-editing technologies such as base editing and prime editing

In addition, the development of derivative editing technologies based on CRISPR-Cas9, including "base editing" and "prime editing," has also attracted considerable attention. Unlike canonical CRISPR-Cas9, these tools achieve accurate single-base conversions, such as A-to-G and C-to-T substitutions, and base transversions. For prime editing, target sequences can be directly encoded

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onto prime-editing guide RNA (pegRNA). The whole reaction does not demand an exogenous DNA donor template, avoids DNA double-strand breaks, and delivers off-target frequencies dramatically reduced relative to wild-type Cas9 [10].

3. Integrated process system for the preparation of gene-edited stem cells

3.1. Clinical outcomes of BCL11A-targeted gene editing therapy (exagamglogene autotemcel, exa-cel)

Phase III clinical trial data confirm that following infusion of edited autologous $CD34^+$ hematopoietic stem cells, over ninety percent (94.2%) of patients with beta-thalassemia achieved complete independence from lifelong transfusion therapy, while sickle cell patients (97%) experienced substantial resolution of recurrent painful vaso-occlusive crises, with hematologic parameters maintained within normal ranges over the long term; concurrently, patient-reported quality-of-life outcome measures encompassing physical, psychological, and social functioning demonstrated sustained improvement [11].

3.2. G-CSF combined with plerixafor for hematopoietic stem cell mobilization and collection

We established as a standardized clinical protocol. Peripheral blood stem cells are initially mobilized and enriched with granulocyte colony-stimulating factor combined with plerixafor, then are subjrcted to precise in vitro gene editing and cryopreservation. After busulfan-induced myelosuppressive preconditioning, the cells are reinfused intravenously, allowing hematopoietic cells to achieve long-term engraftment and differentiation in the bone marrow, and this therapeutic efficacy remains stable over a patient's lifetime [11].

3.3. Clinical manifestation disparities between TDT and SCD patients during the mobilization process and the limitations of mobilization in TDT patients

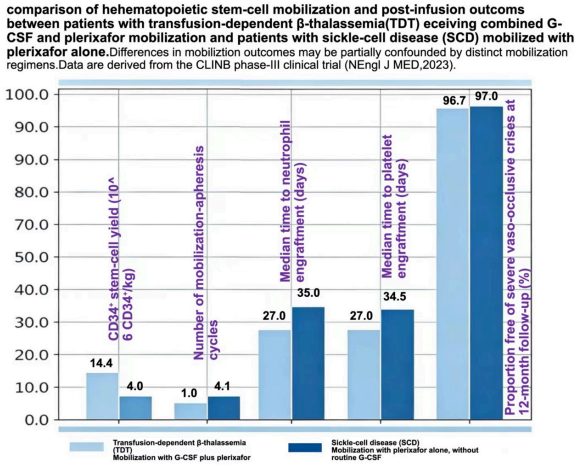


Figure 3. Comparison of hematopoietic stem cell mobilization and transplantation outcomes in patients with β-thalassemia and sickle cell disease

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However, this standardized mobilization regimen is applicable only to patients with β-thalassemia. G-CSF is contraindicated in patients with sickle cell disease, for whom stem cell collection can be performed solely with plerixafor, substantially increasing the complexity of the mobilization procedure and the risk of clinical adverse outcomes. Multidimensional differences exist between TDT and SCD during the mobilization process, as presented in Figure 3.

4. Existing technical limitations, unresolved clinical issues, and next-generation development prospects of gene editing therapies targeting HBG1/HBG2 and BCL11A

Despite the remarkable clinical efficacy, this therapy still faces multiple unresolved research gaps and limitations. First, there is currently no safe, efficient mobilization regimen that does not induce vaso-occlusive crises (VOCs), and no unified strategy exists to balance hematopoietic stem cell collection efficiency with the risk of vaso-occlusion in patients with SCD [12]; optimized preconditioning regimens, preoperative exchange transfusion, and other optimization strategies remain in the exploratory stage. Furthermore, owing to the cellular heterogeneity of HbF distribution, mean HbF levels cannot represent the protective status of all erythrocytes, and the existing clinical follow-up periods are relatively short [5]. The quantitative relationship between HbF concentration thresholds and long-term organ protection as well as symptom recurrence risk has not yet been established [11]. Furthermore, current myeloablative conditioning regimens frequently induce hepatic injury and severe cytopenia, while long-term genotoxicities such as off-target editing and aberrant genomic recombination remain inadequately supported by cohort data spanning over a decade. Moreover, the issue of interpatient heterogeneity in therapeutic response is particularly pronounced, with middle-aged and elderly patients exhibiting significantly lower event-free survival and overall survival rates following treatment compared with adolescent and young adult populations. Concurrently, contemporary clinical trials are characterized by insufficient enrollment of middle-aged and elderly participants and inadequate stratified analyses [12, 13]; Most studies merely report age-related differences in therapeutic efficacy without systematically elucidating the underlying causal mechanisms; the relative contributions of age, cell editing efficiency, and baseline disease course to prognosis remain inadequately characterized [14]. Furthermore, in patients with severe sickle cell disease, the stem cell mobilization phase is prone to precipitating acute complications, and the substantial cost of drug manufacturing constrains the widespread adoption of this therapeutic approach in underdeveloped regions with a high prevalence of hematological disorders.

5. Discussion

5.1. Interpretation of research findings, limitations, and generalizability analysis

Overall data show that editing the HBG1/HBG2 promoter works better for raising HbF levels. This happens because it directly disrupts repressive elements. In comparison, targeting BCL11A has weaker activation efficiency, though its geomic stability id better. Older patients often show weaker therapeutic effects. This might be caused by impaired DNA repair and lower seif-renewal capacity intheir aged stem cells (both propositions are conjectures based on existing findings). We compared nultiple editing targets side-by-side, including new base editing and prime editing tools. It addresses the limitation of earlier reviews that focused on a single target alone, and offers a literature reference for personalized gene therapy regimens in hematological disorders. Even so, clear boundaries remain: stratified samples from middle-aged and elderly populations are relatively scarce, and the

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mechanistic explanation for age-related differences in efficacy still lacks support from basic experimental evidence, which constrains the generalizability of the relevant conclusions.

5.2. Potential optimization strategies

We propose four strategies to address these clinical limitations. First, we recommend propective cohort studies. These trials will help collect more accurate pre-conditioning tolerance data across different age groups and disease subtypes, so that we can establish stratified dosing criteria. Meanwhile, we should keep pushing forward clinical tests on antibody-drug conjugate (ADC) conditioning regimens that remove chemotherapy. The main goal is to see if these new solutions can reduce toxic risks(both acute and long-term). At the same time, we mudt ensure stable engraftment. Third, long-term follow-up data should be integrated to elucidate the quantitative relationships among HbF levels, F-cell distribution characteristics, and long-term organ complications. We also hope to establish standards for evaluating the long-term efficacy of gene editing therapy. Finally, for the SCD patients, we need to create safer and more efficient stem cell mobilization approaches that do not rely on G-CSF. This is important because it solves a common clinical problem that is collecting stem cells from this patient group is often difficult.

6. Conclusion

This paper confirms that CRISPR-Cas9-based editing of BCL11A and HBG targets demonstrates curative potential in the treatment of sickle cell disease and transfusion-dependent beta-thalassemia, with current follow-up data indicating stable treatment effects and high clinical value in specific patient groups. A comprehensive comparison of clinical trial data from the past five years reveals that this therapeutic approach still presents many shortcomings in clinical translation. First of all, therapeutic outcomes exhibit age-stratified disparities. Furthermore, the risk of complications in stem cell mobilization is not same for SCD and TDT patients. Third, next-generation precision editing technologies such as base editing and prime editing still need further clinical validation.

Overall, CRISPR gene-editing therapies are expected to evolve toward greater precision and lower toxicity. Researchers will progressively completing clinical trial validations. Finally, this therapy may become manufactured widely and available to more patients.

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