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Comparison Between Two CRISPR-Cas9 Editing Strategies for Treating B-thalassemia

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Abstract. β-Thalassemia is a kind of single gene inheritance disease caused by HBB gene mutation. Traditional therapies, like lifelong blood transfusions and iron chelation, impose a heavy burden, while allogenic transplantation is limited by the lack of matched donors. Thus, there is an urgent need for curative ways free from donor availability. CRISPR-Cas9 gene editing offers two pathways: directly correcting the pathogenic HBB mutation by homology-directed repair (HDR) to restore adult hemoglobin (HbA), or disrupting the BCL11A erythroid enhancer through non-homologous end joining (NHEJ) to reactivate fetal hemoglobin (HbF) for functional compensation. This article compares these two strategies in five dimensions: molecular mechanisms, treatment process, clinical evidence, safety risks, and economic costs, and offers an assessment based on literature data and mechanistic analysis. Results show that the BCL11A enhancer editing strategy (exa-cel) has already approved for market use, offering broad applicability, contrary to direct HBB repair offering radical treatment limited by low HDR efficiency in hematopoietic stem cells and the risk of off-target effects involving homologous genes. The latter is mainly in preclinical stage. The article argues that it should put priority to establish the HbF-compensatory approach in short term, whereas long-term efforts should advance the radical treatment. Meanwhile, challenges arouse in balancing efficiency, safety, ethics and accessibility while developing gene editing.

Keywords: β-thalassemia, CRISPR-Cas9, HBB gene, BCL11A, fetal hemoglobin

1. Introduction

β-thalassemia is one of the most widely distributed monogenic hereditary diseases. Adult hemoglobin (HbA) is composed of two α-globin chains and two β-globin chains, and α-globin and β-globin are synthesized in proportion simultaneously.

The disease is caused by mutations in the β-globin gene (HBB). These mutations reduce (β^+) or abolish (β^0) the synthesis of β-globin chains. HbA formation is then blocked, and the balance between α- and β-globin is broken. The excess α-globin chains deposit inside red cell precursors, which leads to ineffective erythropoiesis and chronic hemolysis [1]. The disease is common along the Mediterranean coast, in the Middle East, in Southeast Asia and in southern China. The combined

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prevalence of β-thalassemia in mainland China is 2.21%, while carrier rates in high-incidence provinces such as Guangdong and Guangxi are markedly higher (Figure 1) [2].

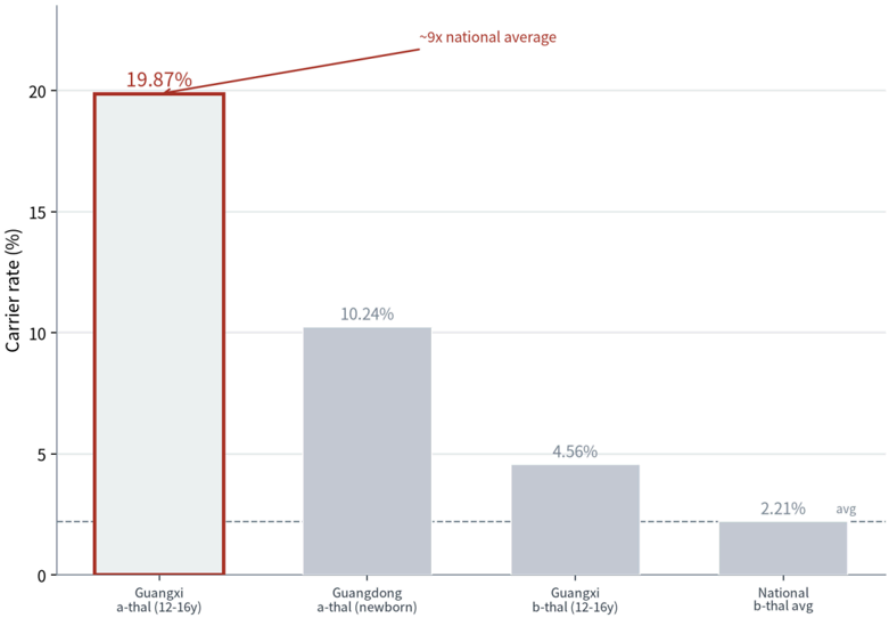


Figure 1. Carrier rate features of β-thalassemia in southern China

Patients with severe thalassemia often need blood transfusions and iron chelation for life, which can damage their organs and cost a lot of money, and a stem cell transplant from a donor is currently the only established cure. Its premise is a healthy donor with matched human leukocyte antigen (HLA). The chance of a matched sibling is only about 25%, and the coverage of unrelated donor registries is limited. Most patients therefore never get the chance of transplantation [1, 3]. Lentiviral gene addition can bypass the donor limit, but random viral insertion carries a risk of insertional mutagenesis. The arrival of CRISPR-Cas9 gene editing has fundamentally changed this situation. It takes the patient's own hematopoietic stem cells as the material. Site-specific editing is finished ex vivo before the cells are infused back. No allogeneic donor is needed, and graft-versus-host disease does not occur. At the same time, the genome can be modified at a precise position. In this way, it removes the two bottlenecks at once, namely the matching and donor scarcity of traditional transplantation and the random insertion of viral vectors [3, 4].

For β-thalassemia, CRISPR-Cas9 has developed in practice along two very different paths. One path directly repairs the pathogenic HBB mutation itself and pursues an etiological cure. The other path does not touch the disease gene. It edits the erythroid enhancer of BCL11A (B-cell CLL/lymphoma 11A). Genome editing downregulates BCL11A expression in erythroid cells, so γ-globin (HBG1/2) is reactivated and fetal hemoglobin (HbF) is produced in large amounts. HbF is assembled from two α-globin chains and two γ-globin chains. It consumes the surplus free α-globin chains and corrects the α/β (α/γ) globin imbalance. This is a functional compensation route, and it improves the pathological phenotype of β-thalassemia. The two strategies differ clearly in action target, DNA repair pathway, mutation adaptability, clinical maturity and safety risk. A systematic review and comparison under the mutation spectrum background of the Chinese population is still lacking, and the choice between clinical and research paths remains confusing.

Given this background, this article systematically compares the two editing strategies in molecular mechanism, treatment process, clinical evidence, safety risk and economic cost, and gives

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a technical judgment. The significance of this article lies in clarifying the strengths and weaknesses of the existing schemes. It also compares candidate targets such as BCL11A, SOX6 (SRY-box transcription factor 6) and HBG (hemoglobin subunit gamma), together with DNA repair pathways such as HDR (homology-directed repair) and NHEJ (non-homologous end joining), and editing tools such as base editing. This comparison may provide a reference for future target screening and individualized program design for patients with different genotypes.

2. Theoretical basis

The HBB gene is located on the short arm of chromosome 11 (11p15.4). The HBB gene provides instructions for making the β-globin chain. This chain combines with the α-globin chain to form adult hemoglobin (HbA), which carries oxygen in the body. More than 300 types of HBB mutations have been reported, including point mutations and small insertions or deletions. In southern China, four common mutations are CD41/42, CD17, IVS-2-654 and −28 (Figure 2) [2]. When the body cannot make enough β-globin, extra α-globin chains build up in developing red blood cells. This can increase oxidative stress, apoptosis and ineffective hematopoiesis [1, 3].

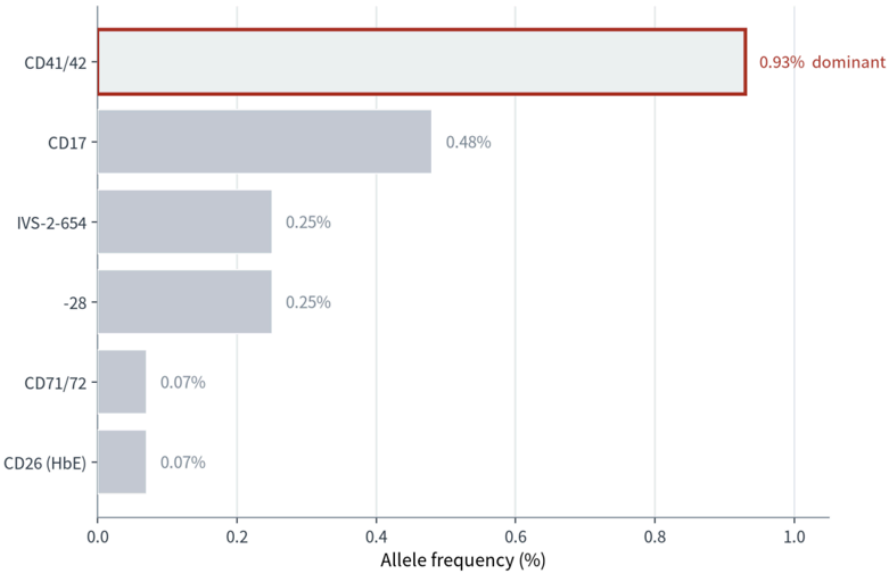


Figure 2. Allele frequencies of common HBB mutations in the Chinese population (data from [2])

CRISPR-Cas9 consists of a guide RNA (sgRNA) and Cas9 nuclease. The sgRNA guides Cas9 to the target site through base complementarity, and Cas9 forms a double-stranded DNA break (DSB) upstream of the PAM sequence. The cell then repairs this break in two ways: non-homologous end joining (NHEJ), which directly connects the two ends of the broken strand. It is highly efficient but randomly introduces small insertions or deletions (indels), so it suits the task of disrupting a regulatory element. Homology-directed repair (HDR) achieves precise base substitution when an exogenous donor template is present, so it suits the correction of a specific mutation. HDR is active only in the S/G2 phase of the cell cycle, while hematopoietic stem cells mostly stay in the quiescent G0 phase. This is exactly the mechanistic root of the efficiency difference between the two strategies [4].

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3. Comparison of the two editing strategies

Table 1 compares the two editing strategies side by side in the dimensions of molecular target, repair pathway, final hemoglobin, mutation coverage, editing efficiency, clinical stage, pivotal evidence, off-target risk and nature of treatment. It provides the basis for the later discussion of the mechanistic differences and clinical translation of the two strategies. As Table 1 shows, the main differences between the two strategies lie in repair pathway, universality, clinical maturity, off-target risk and nature of treatment.

Table 1. Multi-dimensional comparison of the two CRISPR-Cas9 editing strategies

Dimension	Strategy A: HBB correction	Strategy B: BCL11A / HbF	Edge
Molecular target	HBB coding region	BCL11A enhancer (intron 2)	—
Repair pathway	HDR, low in G0 HSPC	✓ NHEJ, ~80% efficient	B
Final hemoglobin	adult HbA	fetal HbF (valid carrier)	—
Mutation coverage	custom per mutation	✓ one product, all mutations	B
Editing efficiency	low in quiescent HSPC	✓ ~80% at target	B
Clinical stage	preclinical / early	✓ FDA & MHRA approved, 2023	B
Pivotal evidence	iPSC proof-of-concept	✓ 91.4% TI12 (32/35), Hb 13.1	B
Off-target risk	higher (HBD/HBBP1)	✓ low (non-coding region)	B
Nature of cure	✓ etiological root-cause cure	functional compensation	A

3.1. Strategy A: direct correction of the HBB pathogenic mutation

This strategy follows the logic of etiological correction (the Strategy A column in Table 1). Autologous CD34+ hematopoietic stem cells of the patient are mobilized and collected. Ex vivo editing takes the patient-specific HBB mutation as the target, and an HDR donor template is used to correct the mutant base precisely. Successfully repaired clones are enriched and then infused back after busulfan myeloablative conditioning, so the repaired stem cells keep differentiating and produce normal HbA [3, 5]. Preclinical studies have verified its feasibility in patient-derived induced pluripotent stem cells (iPSCs). Edited iPSCs carrying the homozygous CD17 (A>T) mutation showed a normal karyotype, kept pluripotency and had no detectable off-target editing. They also recovered HBB expression, showed lower reactive oxygen species levels and gained higher hematopoietic differentiation efficiency. Successful repair has also been reported for mutations common in the Chinese population, including the CD41/42 deletion, the IVS-2-654 splicing mutation, hemoglobin E and the CD39 nonsense mutation [5, 6]. New base editors can even repair the −28(A>G) promoter mutation directly without creating a double-strand break [7, 8].

The outstanding advantage of this strategy is that it restores normal HbA from the etiological level. It is a radical cure in the true sense, and autologous cells carry no rejection risk. Its bottlenecks are equally clear. First, HDR efficiency is low in quiescent hematopoietic stem cells. A customized sgRNA and donor template is needed for each mutation, so the strategy is hard to generalize. Second, HBB sits next to the highly homologous HBD gene and the HBBP1 pseudogene, and low-level off-target base editing has been detected at these sites [8]. At present, most studies of this strategy remain in the preclinical or early clinical stage.

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3.2. Strategy B: editing the BCL11A enhancer to reactivate fetal hemoglobin

Rather than correcting the HBB mutation, this strategy increases fetal hemoglobin (HbF) by disrupting the erythroid-specific BCL11A enhancer. BCL11A is the master repressor that silences the γ-globin genes. After birth, working with co-repressors such as SOX6 and reshaping the three-dimensional chromatin of the β-globin cluster, it drives the switch from fetal HbF ($\alpha_2\gamma_2$) to adult HbA ($\alpha_2\beta_2$) [7, 9]. Within the second intron of BCL11A, researchers found an erythroid-specific enhancer that carries a GATA1 binding site. By using the NHEJ mechanism with CRISPR-Cas9 to knockout this enhancer, the expression of BCL11A was only down regulated in erythroid cells, while remaining unchanged in other tissues. Once the inhibition of HBG was relieved, γ-globin was re-expressed, and the level of HbF increased to compensate for the deficient HbA [10, 11]. In preclinical studies, the editing efficiency at this site reached approximately 80%, and no off-target editing was detected [10].

The clinical candidate drug based on this concept is exa-cel (exagamglogene autotemcel, trade name Casgevy). In an early clinical trial, patients with transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) showed increased HbF levels after receiving the edited cells. Some patients with TDT were able to stop regular blood transfusions. These results were maintained during at least one year of follow-up [10]. The phase 3 CLIMB THAL-111 trial then put the approach on firmer ground: 32 of 35 evaluable patients (91.4%) stayed transfusion-independent for at least 12 consecutive months, mean total hemoglobin reached 13.1 g/dL with HbF at 11.9 g/dL, and edited allele levels stayed stable over time [11]. In 2023 exa-cel won approval from the MHRA in the United Kingdom and then the FDA in the United States, becoming the first CRISPR-based therapy ever marketed [11, 12].

3.3. Comparison and personal judgment

The two strategies differ in four main aspects: mutation coverage, clinical development, safety, and therapeutic goal.

First, the BCL11A editing technology is not affected by the type of HBB mutations carried by patients, so one product can be applicable to all genotypes. In contrast, direct HBB repair targets specific mutations. Second, the two strategies differ in clinical development that one has advanced to late-stage clinical trials, while the other is still at an earlier stage of development. Third, their safety considerations are different. BCL11A enhancer editing targets a non-coding region and mainly relies on NHEJ, while direct HBB repair uses HDR to correct the target sequence. Finally, the two approaches have different therapeutic goals: BCL11A enhancer editing aims to improve the symptoms by increasing HbF levels, while HBB repair aims to correct the underlying genetic defect.

Beyond these four differences, several technical limitations and safety issues also need to be considered.

First, HDR mainly occurs during the S/G2 phases of the cell cycle, while hematopoietic stem cells are often in the G0 phase. This limits the efficiency of HDR-based editing, and sgRNA optimization alone may not solve this problem. Second, NHEJ can introduce different types of insertions or deletions, but these changes may be acceptable when editing a non-coding enhancer. This approach also avoids the need for an external DNA repair template. As a result, it may achieve higher editing efficiency in some cases. Third, base editing and prime editing may be useful alternatives to traditional Cas9-HDR because they can reduce the need for double-strand DNA breaks. However, their effects on the cell cycle and their suitability for hematopoietic stem cells depend on the specific editing system. Base-editing approaches for HBB mutations may enter

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clinical trials in the coming years, but the prediction that they will replace HDR-based treatments within three to five years needs further evidence [8, 13]. Finally, off-target effects should be carefully assessed. Methods such as GUIDE-seq may not detect all off-target changes, and results from laboratory studies may not fully reflect what happens in patients. Long-term follow-up is therefore important for evaluating safety. The proposed follow-up period of ten to fifteen years should be supported by relevant references [14].

4. Ethical safety and economic feasibility analysis

Both of these strategies belong to somatic cell editing, which only modifies the patient's own hematopoietic system and does not be passed on to future generations. The ethical risks are comparable to those of conventional gene therapy, and they have been approved by regulatory authorities. In contrast, germline editing is strictly prohibited because it can genetically modify the human gene pool: in 2018, He Jiankui's editing of the human embryo's CCR5 gene sparked global condemnation, and in 2019, 18 scholars including Lander, Baylis, and Zhang Feng jointly called for a suspension of clinical germline editing in the journal Nature [14, 15]. In terms of safety, strategy A has a higher risk of off-targeting due to its proximity to homologous genes; strategy B has a single target and edits the non-coding region, and the overall clinical safety is good, but multiple edits (such as the simultaneous knockout of BCL11A and SOX6) may cause chromosomal translocations. Future combined strategies must focus on monitoring [12]. Additionally, gene therapy is mostly used for patients over 12 years old, and informed consent from minors is involved. The follow-up time of existing clinical studies is still limited to several years [9, 10], and patients must be fully informed of the uncertainty of the persistence and delayed risks of the therapeutic effect.

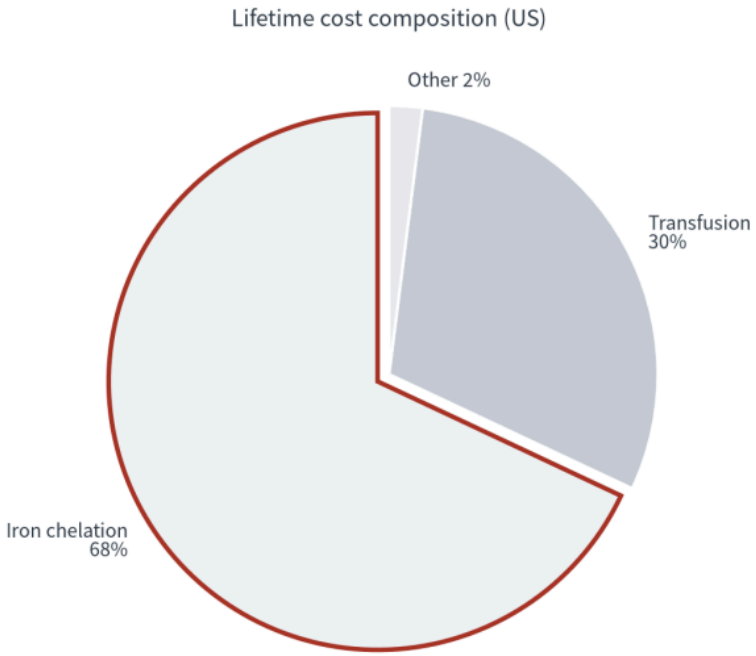


Figure 3. The medical expenses of traditional care versus gene therapy

In terms of direct costs, the total lifetime medical expenses for American patients amount to approximately 5.4 million US dollars, with chelation therapy accounting for 68 % and blood transfusion accounting for 30 % [16] (Figure 3). For adult severe patients in China, the lifetime

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direct costs (not discounted) are approximately 519,000 US dollars, with chelation therapy accounting for 75.6 %, and the Guangdong and Guangxi patients in the study sample accounted for 90.7 % in total, highlighting the regional concentration of disease burden [17]. However, direct costs are just the "tip of the iceberg": a systematic review that included 75 studies showed that patients were absent from work for an average of 15.6 to 35 days per year due to treatment, and the time spent on disease management each day for blood transfusion was as long as 592 minutes. Caregivers were absent from work for an average of 19 days per year and had even higher psychological stress scores than the patients themselves; non-blood transfusion-dependent patients had worse quality of life but were often overlooked; Chinese studies also showed that 44 % of patients had interrupted blood transfusion and 38.7% had interrupted chelation therapy [18].

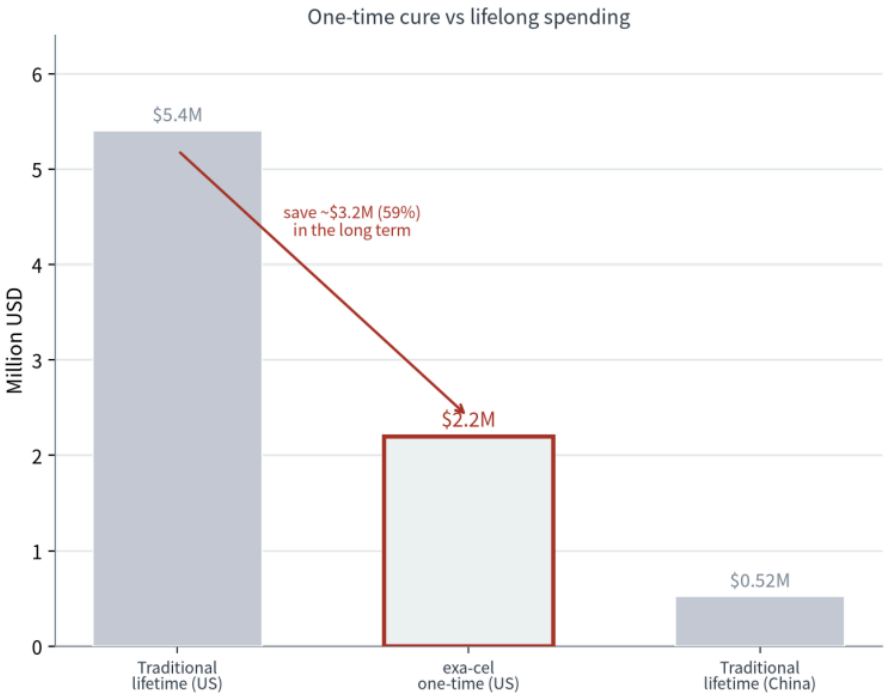


Figure 4. The economics of traditional care versus gene therapy

In terms of gene therapy, Exa-cel is priced at 2.2 million US dollars per course in the United States and 1.65 million pounds in the United Kingdom; the cost-effectiveness analysis shows that its incremental cost-effectiveness ratio is 11.4 million US dollars per QALY (from the payer's perspective) and 5.5 million US dollars per QALY (from the social perspective) [12, 19] (Figure 4). This article believes that assessing solely from the payer's perspective will underestimate the value of gene therapy - it not only saves lifetime medication costs, but also liberates patients and their families from the time shackles of lifelong treatment, restoring productivity, educational opportunities, and mental health, which have significant social value. However, 2.2 million US dollars is still an astronomical figure for developing countries with a high incidence of the disease. The mismatch of "disease burden in the south and high-priced drugs in the north" reflects the structural inequality of the global health system and requires the promotion of fairness and accessibility through technology transfer, differentiated pricing, and regional joint procurement.

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5. Discussion and future perspective

Based on the above comparison, this paper believes that in the short term, the mature HbF compensation scheme should be promoted first, and precise HBB repair should be regarded as the long-term research direction: exa-cel has sufficient evidence, good safety, and universality, and can immediately serve a wide range of patients with diverse mutations; direct repair is limited by efficiency and cost, and awaits the maturity of new editing tools. In terms of patient selection, stratified treatment should be adopted: patients with severe conditions and limited finances should be prioritized for exa-cel; those with specific mutations that have mature repair solutions and are willing to take risks can participate in repair-based clinical trials; elderly or patients with organ damage should be carefully evaluated for myeloablative toxicity. Future directions include: combination of HbF induction drugs and gene editing, multi-target editing such as BCL11A and SOX6 (be cautious of chromosomal translocations), auxiliary strategies to reduce free α chains, and in vivo editing and non-myeloablative pre-treatment to expand the applicable population [6, 12].

From the perspective of technological evolution, gene editing is currently in the critical stage of moving from "0 to 1" feasibility verification to "1 to 100" optimization and popularization: on the editing tools, high-fidelity Cas9, base editing, and lead editing will gradually reduce off-target and chromosomal abnormalities; on the delivery methods, the complex in vitro editing - myeloablation - reinfusion process is expected to be simplified by in vivo editing; on the indications, it will expand from transfusion-dependent to non-transfusion-dependent and more single-gene genetic diseases. This paper always holds humanistic expectations: β-thalassemia should not be a "predestined burden" for patients. CRISPR has for the first time given humans the ability to rewrite their own genetic code, turning the fate written in DNA into a problem that can be intervened after the fact; from the establishment of CRISPR principles to the approval of exa-cel, it took only about ten years, and the technological acceleration is worthy of optimism. This paper also objectively explains the research limitations: this paper conducts theoretical analysis based on public literature, lacks first-hand clinical data, does not subdivide genotypes and age subgroups, and the economic data mostly comes from Europe and the United States, and the long-term safety still needs to be verified through follow-up for more than ten years.

6. Conclusion

CRISPR-Cas9 provides two fundamentally different pathways for treating β-thalassemia: direct repair of HBB belongs to a root-cause cure but is limited by HDR efficiency and off-target risks, and the clinical translation is lagging; BCL11A enhancer editing activates HbF, which is a functional compensation, but it has been approved for market use, achieving independent blood transfusion for 91.4% of patients and having the universality to cover all mutation types. The divergence between the two essentially lies in the differences in the two repair pathways (NHEJ and HDR), as well as the characteristics of the two types of targets (non-coding regulatory regions and coding regions). This article concludes: In the short term, the HbF compensation scheme should be prioritized for promotion; in the long term, base editing and lead editing should be relied upon to advance precise repair; the core proposition of gene editing has shifted from "whether it can be achieved" to "how to achieve a balance between efficacy, safety, ethics, and accessibility", and the scientific selection of targets and the iterative improvement of editing tools are the key paths to achieving this balance. With the establishment of technology maturity, cost reduction, and fair access mechanisms, β-thalassemia is expected to transform from a "born constraint" to a "disease that can be cured in the future", and CRISPR will more widely and accurately benefit more patients with genetic diseases.

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