Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

Recent Advancements in Ex Vivo CRISPR/Cas9 Gene Editing for Hemoglobinopathies: A Systematic Review of Clinical and Preclinical Progress

Ashwaq Raheem Nazzal[1] , Rana Khalaf Abdulsamad[2] , Tamadhir A. Al Hamed[3] , Hazim T. Thwiny[4]

Email : ashwaq.raheem@uobasrah.edu.iq [1] , hazim.thwiny@uobasrah.edu.iq [4]

[1,2,3] College of Veterinary Medicine, University of Basrah, Basrah, Iraq

[4] AlZahraa college of medicine, University of Basrah, Basrah, Iraq

Abstract

This systematic review provides an overview of recent developments in ex vivo CRISPR/Cas9 applications to gene editing to address hemoglobinopathies, predominantly sickle cell disease and 1 -thalassemia. The papers published between 2020 and 2025 were evaluated and discussed molecular techniques, such as non-homologous end joining (NHEJ), homology-directed repair (HDR), base editing, and prime editing. BCL11A enhancer editing and fetal hemoglobin reactivation are the most advanced strategies clinically and with current evidence. The other factor leading to these promising outcomes in preclinical research is the possibility of base editing and prime editing to allow a more precise genetic correction, with less risk of causing a double-strand DNA break. Many important challenges still need to be solved, including off-target effects, genetic mosaicism, delivery efficiency, and clonal hematopoiesis after treatment, when mutations occurring in HSCs confer a proliferative advantage to those cells, resulting in malignancies− the risk of genotoxicity due to the high cost and need for special treatment centers. Conclusion: ex vivo CRISPR-based therapy is a powerful new therapeutic option in hemoglobinopathies that we hope to implement clinically, but still, it is hindered by hurdles in long-term safety and in translational considerations of access and cost .

Keywords :CRISPR/Cas9, hemoglobinopathies, sickle cell disease, 2 -thalassemia, fetal hemoglobin.

الملخص

تقدم هذه المراجعة المنهجية نظرة عامة على التطورات الحديثة في تطبيقات تقنية كريسبر/كاس9 خارج الجسم الحي لتحرير الجينات بهدف معالجة اعتلالات الهيموجلوبين، ولا سيما فقر الدم المنجلي والتلاسيميا الأولى. وقد تم تقييم ومناقشة الأبحاث المنشورة بين عامي 2020 و2025، والتي تناولت التقنيات الجزيئية، مثل الربط غير المتجانس للنهايات (**NHEJ**)، والإصلاح الموجه بالتماثل (**HDR**)، وتحرير القواعد، والتحرير الأولي. ويُعد تحرير مُحسِّن **BCL11A** وإعادة تنشيط الهيموجلوبين الجنيني من أكثر الاستراتيجيات تقدماً من الناحية السريرية، وذلك استناداً إلى الأدلة الحالية. ومن العوامل الأخرى التي تُسهم في هذه النتائج الواعدة في البحوث ما قبل السريرية، إمكانية استخدام تحرير القواعد والتحرير الأولي لإجراء تصحيح جيني أكثر دقة، مع تقليل خطر حدوث كسر في سلسلتي الحمض النووي. لا تزال هناك العديد من التحديات الهامة التي تتطلب حلولاً، بما في ذلك التأثيرات الجانبية، والتنوع الجيني، وكفاءة التوصيل، وتكوين الدم النسيلي بعد العلاج، حيث تُكسب الطفرات التي تحدث في الخلايا الجذعية المكونة للدم هذه الخلايا ميزة تكاثرية، مما يؤدي إلى الأورام الخبيثة، بالإضافة إلى خطر السمية الجينية نتيجة التكلفة العالية والحاجة إلى مراكز علاج متخصصة. الخلاصة: يُعد العلاج القائم على تقنية كريسبر خارج الجسم الحي خيارًا علاجيًا جديدًا وفعالًا لأمراض الهيموجلوبين، ونأمل في تطبيقه سريريًا، ولكنه لا يزال يواجه عقبات تتعلق بالسلامة على المدى الطويل، واعتبارات التطبيق السريري المتعلقة بإمكانية الوصول والتكلفة.

الكلمات المفتاحية : CRISPR/Cas9، أمراض الهيموجلوبين، مرض الخلايا المنجلية، الثلاسيميا من النوع 2، الهيموجلوبين الجنيني.

Introduction

1.1 Genetic and Clinical Burden of Hemoglobinopathies.

Hemoglobinopathies are a collection of inherited disorders that impact on the structure or synthesis of hemoglobin, the molecule that carries oxygen in red blood cells. Mutations in the globin g enes are the main cause of these disorders and affect the synthesis of normal hemoglobin chains, either through qualitative defects (structural defects) or quantitative deficiencies in their formation. Among them, the most frequent and most clinically significant forms are sickle cell disease (SCD) and β-thalassemia which cause significant morbidity and mortality on the global level [1].

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

The pathophysiology of sickle cell disease is a one-nucleotide deletion in the HBB gene which changes hemoglobin S (HbS). It leads to a single amino acid replacement (position 6), which significantly changes the biochemical characteristics of Hemoglobin of Hemoglobin A. Each time HbS molecules bind deoxygenated hemoglobin, they polymerize, distorting the red blood cells into a rigid crescent-moon, or sickle, shape-this loss of normal morphology leads to reduced cell deformability, which disrupts microcirculatory flow [2].

Sickling of red blood cells has multidimensional and systemic pathophysiological effects. Sickle cells stick to the vascular endothelium and block small blood vessels, resulting in vaso-occlusive events. Later, these lead to a condition of ischemia, tissue damage and the serious pain crises that define the disease. Moreover, sickling and unsickling do cause membrane damage, which in turn causes hemolysis and chronic anemia [3].

On the other hand, the β-thalassemia is caused by mutations that inhibit or prevent the production of 4 chains of -globin. This defect in the assembly of erythroid precursors in the marrow leads to ineffective erythropoiesis and to the destruction of erythroid precursors in the marrow. The surplus α-globin chains accumulate into dissolvable and insoluble forms that destroy the erythroid progenitors in the course of developing red blood cells, and result in severe anemia and requires chronic transfusion therapy [4].

Throughout the entire night, hemoglobinopathies are the most common cause of disease, and a very high mortality rate is seen in a few geographic areas (sub-Saharan Africa, the Middle East, South Asia, and the Mediterranean). These areas have a high malaria burden due to genetic, environmental, and evolutionary factors, including malaria protection due to having specific hemoglobin variants. This implies that there are millions of individuals on the planet who are impacted, even healthcare systems [5].

Hemoglobinopathies cause a myriad of complications in patients despite effective treatment. These include chronic anemia, recurrent infections, organ damage and stroke, and pulmonary hypertension with greater morbidity and mortality. The complications concern the background history and genetic background of people, as well as environmental issues, social determinants of health, and the access of healthcare resources [6].

These disorders are also a great socioeconomic burden, both in the clinical and economic sense. They require more hospitalization, medication and transfusions in their whole life. These medical expenses are huge and more so in the low to middle income nations that have low resources [6].

Therefore, hemoglobinopathies not only present a clinical and genetic challenge, but are also an important global health burden. Existing therapies primarily offer long-term control, but not elimination and effective regular regimens of treatment have limits to providing functional bioactivity- highlighting an urgent need to develop novel curative modalities, including genome editing technologies capable of offering a direct means of intervention on the cause of disease [6].

1.2 Constraints in Hematopoietic Stem Cell Transplantation and Conventional Gene Therapy.

The long-held theory of hemoglobinopathies is that hematopoietic stem cell transplantation (HSCT) can be a potentially curative treatment. It operates by transplanting donor substitute and functional stem cells that have the potential to produce normal hemoglobin in lieu of the defective hematopoietic stem cells of a patient. When successful, HSCT can have a long-term disease remission, or even a permanent cure [7]. While HSCT offers therapeutic promise, it is associated with key limitations. The most demanding is that donor must be provided preferably a sibling who is compatible in the

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

HLA. But the vast majority of patients do not have access to such donors, and this treatment is not accessible by most of the affected people in the world [8].

The other significant limitation is that graft-verified-host disease (GVHD) may occur in which transfused donor immune cells can proliferate and assault the recipient tissues. GVHD not only has mild forms but also life-threatening forms, and it is still one of the most dangerous complications of transplantation. It can as well be incompatible with the graft, particularly in patients who have undergone multiple transfusions or who are immunologic sensitive [9].

There are extra risks of pretransplant conditioning regimens. These regimens are usually chemotherapy or radiation which is used to kill the cancer and other cells in the bone marrow of a patient. They are necessary to successful engraftment, but they also result in significant toxicity, such as infertility, organ damage and increased risk of infection [10].

Conventional gene therapy has also been developed as an alternative strategy, entailing the delivery of a functional -globin gene into autologous hematopoietic stem cells with the help of viral vectors. The strategy does not require a donor and decreases the chance of immune rejection. There have been positive results in the trials, which have shown a decrease in transfusion needs and hemoglobin levels [11].

Gene therapy, on the other hand, involves a viral vector that is more complex. Random insertion of the therapeutic gene in the genome may trigger oncogenes or silence tumor suppressor genes leading to insertional mutagenesis. Uneven therapeutic benefit could also be a result of varying expression of the gene insertion [11].

Another limitation is the complexity and high cost of the manufacturing process of gene therapy. The cost of treatment is expensive as well as scaled down by the requirement to have specialized production facilities to make the viral vectors and process patient-derived cells [9].

These shortcomings demonstrate the necessity of more specific, effective and safer treatment options. But in the case of gene therapy of blood diseases, there is a huge obstacle; the actual or possible introduction of genes into other genetic loci which can lead to downstream effects of malignant transformation. Genome editing technologies offer an open entry point and prominent amongst this category have so far been CRISPR/Cas9 which overcomes the challenges of random gene(s) insertion by targeting modification of specific regions of interest within the genome of the patient, and emerged in more recent years, laboratory-developed vectors (gets around the problems associated with delivering such types elsewhere in former cases) [7].

1.3 Historical Background of CRISPR/Cas9 in Hematologic Disorders.

The CRISPR/Cas9 system was first identified as part of the bacterial adaptive immune response, which protects against viral infections. Scientists later adapted this system into a powerful genome-editing instrument that could be used to make targeted changes to the DNA with precision [12].

CRISPR/Cas9 adaptation: Adaptation of CRISPR/Cas9 to mammalian cells has been a breakthrough in the history of genetic engineering. Researchers can target the Cas9 nuclease to any locus in the genome by designing complementary guide RNAs that are specific to that DNA sequence [5].

CRISPR/Cas9 has been the most typical method in this field of the study in hematologic disorders due to the more accessible hematopoietic stem cells. They are easy to isolate off patients, can be edited ex vivo, and reinfused after genetic repair, making them natural targets to therapeutic genome editing [7].

The first demonstration of CRISPR/Cas9 usage

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

in hemoglobinopathies was the repair of the sickle mutation itself. However, the challenges linked to homology-directed repair made researchers refer to other ways such as the reactivation of fetal hemoglobin [8].

One of the breakthroughs in this field has been the identification of BCL11A which is an effective repressor of the fetal hemoglobin expression. Crisis PR/Cas9-mediated genetic disruption of the erythroid-specific enhancer of BCL11A resulted in significant upregulation of fetal hemoglobin; thus, providing a compensatory mechanism when adult hemoglobin production fails [1].

It is a particularly good method used in preclinical and clinical trials, leading to better patient outcomes. A novel paradigm of hemoglobinopathy treatment with the ability to induce fetal hemoglobin without alteration of the 3-globin gene per se [11].

Over time, increasingly advanced CRISPR technologies were created, such as base editing and prime editing. What prevents genome instability is the absence of double-strand breaks, which allows targeted conversion of nucleotides [8].

CRISPR/Cas9-based treatments are rapidly emerging in clinical trials, and they are going to leave the bench beside the bedside. Together, this development highlights the ethical and practical implications of genome editing when it comes to the treatment of hereditary blood disorders [12].

2. Molecular Underpinnings of gene editing approach.

2.1 NHEJ-Mediated Editing: Reactivation of Fetal Hemoglobin.

The primary DNA repair pathway in mammals is non-homologous end joining (NHEJ), which is a component of CRISPR/Cas9 genome editing. Having no homologous template, NHEJ repairs the Cas9 nuclease-induced DNA double-strand breaks. However, this mending process is prone to errors and frequently leads to an insertion or deletion (indel) at the cut site [13].

Notably, the error-prone character that NHEJ exhibits in therapeutic genome editing can be used to the advantage of crippling gene function. Otherwise, researchers can silence a gene or a regulatory element that is among the pathology of a disease using NHEJ. This is particularly useful with hemoglobinopathies, where modifications of the gene function may be used to provide better therapy [14].

In this regard, transcription factor BCL11A is a prime target since it suppresses the expression of fetal hemoglobin (HbF) after birth. When developing normally, HbF is replaced by fetal hemoglobin (HbA). Nevertheless, induction of HbF in adults greatly reduces the clinical manifestations of sickle cell disease and β-thalassemia [15].

Molecular disruptions of the BCL11A erythroid-specific enhancer by CRISPR/Cas9 decrease transcription of this repressor in red blood cell progenitors. Because hematopoietic tissues are the only ones targeted, this specific disruption of hematopoietic tissues can increase HbF production but does not compromise BCL11A functions in other tissues, severely limiting its systemic effects [16].

This approach is clinically relevant, with the examples of patients with hereditary persistence of fetal hemoglobin (HPFH), who have naturally high levels of HbF and benign disease phenotype. However, since the approaches based on NHEJ are effective in curing infections by merely approximating this natural condition using genome editing, we should be careful when referring to these therapeutic approaches as curative [17].

Such NHEJ-based editing is one of the most efficient repair mechanisms compared with other repair mechanisms, such as homology-directed repair (HDR). The reason why NHEJ is highly effective in noncycling hematopoietic stem cells is due to the fact that it is active in the entire cell cycle [18].

But this method is limited too. Being a random event, the indel formation may lead to variable

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

editing effects and even cause alteration of adjacent regulatory factors. Too much editing could also impact the long-term health or development of a cell [19].

Nonetheless, despite these challenges, however, NHEJ-mediated editing is now the most clinically advanced approach to the management of hemoglobinopathies. It is the basis of currently used and tested CRISPR-based therapies because it is highly efficient, relatively easy to use, and has been shown to be highly successful in clinical trials [20].

2.2 Editing via HDR-Mediated Editing: Accurate Mutation Repair.

HDR (homology-directed repair) is a highly specific DNA repair pathway that allows precise repair of mutations. Unlike NHEJ, HDR involves the use of a homologous donor DNA template which guides repair and allows specific nucleotide modifications at the target site [21].

HDR has been prompted by the requirement to correct a mutation in the β-globin gene directly when it is pathogenic. This could imply in the case of sickle cell disease, the replacement of the mutated HbS allele by the wild type (normal) HbA sequence, and can therefore be made to restore normal hemoglobin activity [22].

Specificity of HDR offers an attractive therapeutic strategy that does not alter the native regulatory conditions of the gene. In this way, it ensures that the corrected gene gets expressed at physiological levels yet prevents abnormal expression of the gene/non-specific effects [23]. Conversely, HDR is not as efficient as NHEJ, specifically in the hematopoietic stem cells. This is because HDR is only permissive at certain points in the cell cycle, that is at S and G2 stages, when a sister chromatid is available to serve as a template to repair [24].

One of them is the competition between HDR and NHEJ. NHEJ is also often preferentially used by cells, resulting in unwanted indels as opposed to site-specific repair in the event that a donor template is present. In the same manner, there are further restrictions that are created due to the acquisition of donor templates. The target templates may be administered as single or double strands of DNA or as viral vectors, each with its own limitations in terms of efficiency, toxicity and immunogenicity [25].

Nevertheless, in spite of these obstacles, the efficiency of HDR has increased tremendously in the past few decades. The donor design, cell cycle synchronization, and the introduction of chemical NHEJ inhibitors have all been demonstrated to increase the efficiency of generation of accurate edits. Although HDR-based methods are not as advanced yet in the clinic as NHEJ-based methods, they nevertheless are of great interest. They have been considered as a pillar to curative therapy of hemoglobinopathies in the future [26].

2.3 Next-Generation Tools: Base Editing and Prime Editing.

The shortcomings of the double-strand break method of genome-editing using the CRISPR/Cas9 system have motivated the development of next-generation genome-editing methodologies. Among these, base editing and prime editing are innovations, which means that physically modified DNA in the double-strand breaks is provided, which is a less disruptive modification. Base editing allows one base to be directly converted into another, without producing double-strand breaks. It may accomplish this by catalytically inactivating a Cas9 protein to a deaminase, which is an enzyme that chemically edits specific bases in the DNA at the target site [27].

Base editing can also be applied to hemoglobinopathies where engineered mutations of natural bases are expected to target regions with elevated production of fetal hemoglobin. To illustrate this, mutations in the γ-globin promoter can erase the binding sites of

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

the repressors or may introduce the binding sites of the activators, thus increasing the level of HgF. However, there are certain limitations of base editing. It can only repair certain types of nucleotide changes (transitions) and therefore not all disease mutations can be repaired directly. In addition, any non-specific mutations in bystanders could be detected in instances where there were two or more bases that are targetable [28].

Conversely, prime editing is a general technique, able to form any base exchange, and small insertions and removals. It uses a Cas9 nickel fusion protein with the reverse transcriptase enzyme, along with a special RNA guide known as the prime editing guide RNA (pegRNA). This system allows much more precise search-and-replace edits to be performed without needing to break the two strands or having to obtain a donor DNA template. In theory, prime editing can directly correct the sickle mutation and thus is an immensely appealing therapeutic option [29].

3. Key Technical Challenges

3.1 Genetic Mosaicism

Genomic mosaicism is a significant barrier to the field of genome editing, and denotes the occurrence of multiple genetically distinct cell lineages in an individual. What nonuniform editing entails in terms of CRISPR/Cas9 DNA is that cells are mosaic when not all the cells have been edited. Particularly, ex vivo editing of hematopoietic stem cells to generate a pure corrected cell population is of great significance here. This might lead to edited and unedited cells, should editing be inefficient or at other time points [30].

Among the numerous causes of mosaic is the delay in editing after cell division. As CRISPR elements may be inherited by daughter cells, with varying CRISPR outcomes, heterogeneity is possible. This is also contributed by variability of the DNA repair pathways. A certain proportion of cells will be successfully edited, whereas other cells might have indels or no change [31].

Mosaicism can limit therapeutic efficacy since unedited or partially edited cells may continue to synthesize defective hemoglobin. Therefore, the proportion of edited cells that can be harvested is a key parameter to clinical efficacy. Newer developed advanced techniques, including single-cell sequencing, enable evaluation of the results of editing at the single-cell level. The methods provide important data on the levels and occurrence of mosaicism [32]. Some of the methods that are under investigation to help reduce mosaicism would be to optimize the delivery methods, improve editing efficiency, and do continual screening of successfully edited cells before they can be reinfused into patients. And in fact, eradication of genetic mosaicism is a precondition to fulfilling both consistency and durability of therapeutic effects related to CRISPR-mediated solutions [33].

3.2 Off-Target Effects

One of the biggest safety concerns of genome editing was still off-target effects. These are where the CRISPR/Cas9 system introduces unwanted mutations into regions of the genome that are related, but not identical, to the target sequence. Accidental insertions and deletions can be especially harmful when they affect genes that control the cell cycle, repair DNA damage, or prevent cancer. Even the low frequency off-target events at stimulating doses could result in long-term risks in real-world-based clinical use [34].

The guide RNA mainly offers the specificity of CRISPR/Cas9 that defines the binding site on the genomic sequence that is offered by the specific guide RNA. Nevertheless, even off-target sites may be cleaved as a result of partial base pairing between the guide RNA and the target. Several methods have been devised to detect the off-target effects and mitigate this issue. CIRCE-seq is an extremely potent biochemical technique that allows detecting genome-wide sites of off-target cleavage with

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

extreme sensitivity [35].

The other significant methodology, besides experimental methods, is the use of computational methods to predict off-target sites based on sequence similarity or thermodynamic properties. Such anticipations help in the designing of more precise guide RNA. The existence or lack of off-target mutations may frequently need experimental validation, such as through deep sequencing. The accuracy of detection and risk assessment is improved by this hybrid approach [36].

Moreover, by further improving CRISPR technology, high-fidelity Cas9 variants with low off-target activity were developed. These synthetic enzymes have the ability to enhance specificity without necessarily losing a significant portion of efficiency. Reducing off-target effects is crucial in ensuring the safety of genome editing in the clinic. This is indeed a challenge but constant advancement in detection, predictability and enzyme design are mitigating the challenge [37-41].

3.3 Delivery Efficiency

Effective transfer of genome-editing reagents to target cells is an essential predeterminer of the success of CRISPR-based therapies. This is particularly hard to do with gainful delivery that maintains cell viability in hematopoietic stem cells. Different modes of delivery have been evolved, and each mode has its advantages and disadvantages. Viral vectors that are effective and potentially cause no issues in immunogenicity and genomic integration are lentiviruses and adeno-associated viruses (AAV) [42-44].

It is believed that, in comparison to viral delivery systems, non-viral delivery systems including lipid nanoparticles are safer since they are less likely to cause insertional mutagenesis. They are however less efficient compared to viral methods in stem cells. The gold standard of editing hematopoietic stem cells is ex vivo electroporation. It is based on treating electric pulses to make the cell membrane temporarily permeable, allowing CRISPR components, including Cas9 ribonucleoprotein complexes, to enter the cells [45].

A benefit of electroporation is that it can be used to deliver CRISPR components in a more transient manner, reducing the time that nucleases are active, and thus minimizing off-target activity. However it can also cause cell stress or decrease viability. The choice of delivery mode may greatly influence the efficiency of editing and cell survival, and, eventually, therapeutic outcome. This leads to the fact that nowadays the primary subject of research is optimization of delivery conditions [46].

New approaches are attempting to maximize delivery rates and reduce toxicity. These are recently developed nanoparticles, improved electroporation regimens, and targeted delivery methods. Finally, when an effective, safe and scalable delivery system of genome-editing tools is developed, the successful validation of CRISPR-based therapies can only be translated into bench-to-bedside therapies [47-50].

4. Methodology

The research was a systematic review evaluating the recent developments in ex vivo CRISPR/Cas9-based gene-editing approaches to hemoglobinopathies, with a specific focus on sickle cell disease and β-thalassemia. Based on an article cycle of 2020-2025, relevant literature was obtained based on major electronic repositories, namely PubMed, Scopus, and Web of Science.

The search strategy used the keywords CRISPR/Cas9, hemoglobinopathies, sickle cell disease, 2-thalassemia and gene (both gene editing, base editing, and prime editing). The search was narrowed down using the search operators of Boolean algebra (AND, OR), so as to include as many studies in the literature review as possible.

The lack of gene editing technologies, and thoroughness of this review: In this scoping review, inclusion criteria included peer-

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

reviewed original articles, clinical trials, and review papers written in English about GEs found in studies of hematologic disorders, and prioritizing those which addressed ex vivo editing of hematopoietic stem cells and clinical translation. The exclusion criteria comprised conference abstracts that lacked full text, non-English publications and studies that were published prior to 2020 but were not identified as highly influential.

Following screening of titles and abstracts, full-text analysis of the data was done on all the selected studies to determine quality and relevancy. The included studies on editing strategies (NHEJ, HDR, base editing, and prime editing), efficiency, safety, off-target effects, and clinical outcomes had their data extracted.

The analysis of the data was done based on the general themes of this review: molecular mechanisms, technical challenges, preclinical models, and clinical applications. Findings were systematically synthesized to outline the prevailing situation of CRISPR technologies in diagnosing and treating disease.

5. Results and Discussion

This evidence suggests that ex vivo CRISPR-based gene editing may be a safe and potentially curative intervention to sickle cell disease (SCD) and β-thalassemia. Although the clinical evidence largely supports the BCL11A enhancer editing and fetal hemoglobin reactivation methods, preclinical studies focus on their potential in the future as base and prime editing. However, safety concerns, such as off-target effects, clonal hematopoiesis, and long-term genotoxicity should be constantly monitored.

Table 1. Summary of Selected Studies on Ex Vivo CRISPR-Based Gene Editing Strategies for Hemoglobinopathies

Study	Design / Type	Main focus	Key results
Frangoul et al. [4]	Clinical trial	CRISPR-Cas9 editing for sickle cell disease and β-thalassemia	Showed early clinical success of CRISPR-Cas9–edited autologous hematopoietic stem cells, with increased fetal hemoglobin and clinical improvement in both diseases.
Frangoul et al. [5]	Clinical trial	Exagamglogene autotemcel for severe sickle cell disease	Demonstrated marked reduction or elimination of vaso-occlusive crises after treatment, supporting clinical efficacy of BCL11A enhancer editing.
Locatelli et al. [9]	Clinical trial	Exagamglogene autotemcel for transfusion-dependent β-thalassemia	Many treated patients achieved transfusion independence with sustained fetal hemoglobin production.
Sharma et al. [31]	Clinical study	Editing HBG1/HBG2 promoters	CRISPR-Cas9 promoter editing increased fetal hemoglobin production and supported its potential as a therapeutic strategy for sickle cell disease.
Fu et al. [18]	Clinical/translational study	BCL11A enhancer editing	Confirmed that BCL11A enhancer disruption can reactivate fetal

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

Study	Design / Type	Main focus	Key results
			hemoglobin and improve β-hemoglobinopathy phenotypes.
Lattanzi et al. [7]	Preclinical study	β-globin gene correction in HSCs	Demonstrated potential for durable correction of the β-globin gene in human hematopoietic stem cells for sickle cell disease.
Uchida et al. [39]	Preclinical study	Gene correction of CD34+ cells	Showed edited CD34+ cells could engraft in xenograft mice and non-human primates, supporting translational feasibility.
Newby et al. [10]	Preclinical study	Base editing for sickle cell disease	Base editing corrected the sickle mutation and rescued disease features in mouse models without causing double-strand DNA breaks.
Zeng et al. [12]	Preclinical study	Base editing of human HSCs	Demonstrated therapeutic base editing of human hematopoietic stem cells with a promising efficiency and safety profile.
Liao et al. [8]	Preclinical study	Adenine base editing in HSCs	Showed improved therapeutic adenine base editing in human hematopoietic stem cells, supporting future precision-editing applications.
Cancellieri et al. [3]	Bioinformatics study	Off-target prediction	Introduced CRISPRitz as a rapid tool for identifying potential CRISPR off-target sites, improving safety assessment.
Liggett et al. [22]	Observational/genomic study	Clonal hematopoiesis in sickle cell disease	Showed that clonal hematopoiesis is an important safety consideration in patients receiving stem-cell-based gene therapies.

Table 2 demonstrated that genotypic correction of 0 -thalassemia, sickle cell or increased fetal hemoglobin revealed a range of moderate to high levels of editing efficiency with a significant increase in the expression of the corresponding globin and either fen crude sickling activity following targeted gene modification. In clinical studies, significant improvements in patient outcomes were evident such as transfusion independence in β-thalassemia and predominant mitigation or abolition of vaso-occlusive crises in sickle cell disease. Preclinical research studies also supported the potential therapeutic use of base editing and promoter editing that highlighted the improvement of hemoglobin expression and reduced the expression of disease-related cellular defects.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

Table 2. Results of CRISPR-based and base-editing therapeutic approaches to hemoglobinopathies.

Study	Type	Disease / Model	n	Editing technique	Editing efficiency %	Main quantitative outcome	Clinical / functional response	Off-target / safety
Frangoul et al. 2021	Clinical / preclinical	SCD and β-thalassemia	2 patients + 10 donors	CTX001 / BCL11A editing	80 ± 6 preclinical; 68.9–82.6 clinical	HbF increased from 10.5 ± 5.2% to 29.0 ± 10.8% in edited cells; patient HbF reached 13.1 g/dL and 43.2%	Transfusion independence in TDT patient; VOC eliminated in SCD patient	No evidence of off-target editing
Frangoul et al. 2024	Clinical	Severe sickle cell disease	44	Exa-cel / BCL11A editing	BM CD34+ 86.1 ± 7.5	HbF increased from 5.4 ± 3.9% to 43.9 ± 8.6% at 6 months	29/30 patients VOC-free ≥12 months; 97%, P<0.001	No graft failure or cancer
Locatelli et al. 2024	Clinical	Transfusion-dependent β-thalassemia	52	Exa-cel	-	Mean HbF during transfusion independence: 11.9 g/dL	32/35 patients transfusion independent; 91%, P<0.001	No deaths or cancers
Sharma et al. 2023	Clinical / preclinical	Sickle cell disease	3 patients + donors	HBG1/HBG2 promoter editing / OTQ923	80.5 ± 9.8 healthy; 85.8 ± 14.7 SCD donors	HbF increased to 19.0–26.8% in treated patients	Improved HbF and hemoglobin; VOC events still occurred	No OTQ923-related adverse events
Fu et al. 2022	Clinical	Transfusion-dependent β-	2	BCL11A enhancer editing	85.46 / 89.48	Hb increased from 8.2 and 10.8	Both patients achieved transfusio	No notable side effects

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

Study	Type	Disease / Model	n	Editing technique	Editing efficiency %	Main quantitative outcome	Clinical / functional response	Off-target / safety
		thalassemia children				g/dL to 15.0 and 14.0 g/dL	n independence >18 months	
Lattanzi et al. 2021	Preclinical	SCD CD34+ HSPCs	-	β-globin gene correction	Up to 60	20% gene correction after transplant into mice	Supported therapeutic threshold and phase 1/2 trial initiation	No genotoxicity or tumorigenicity
Uchida et al. 2021	Preclinical	SCD CD34+ cells / xenograft / primates	1–6 / 3 donors	βS-to-β correction	43–54 in vitro; 10–38 at 24 weeks	β-globin protein reached 81–85%; human engraftment 8–29%	Edited cells engrafted in mice and primates	Off-target <1% at 24 weeks
Newby et al. 2021	Preclinical	Human SCD HSPCs and mouse model	3 donors; 6 edited / 6 control mice	Adenine base editing	80 ± 2.1 human HSPCs; 44 ± 11 post-transplant mice	βS reduced from 87 ± 1.3% to 17 ± 3.0%; sickling reduced from 47.7% to 16.3%	Hematologic rescue in mouse model	No predicted clinical relevance of off-targets
Zeng et al. 2020	Preclinical	Human CD34+ HSPCs	-	CBE / BCL11A enhancer base editing	-	HbF induction in erythroid progeny	Sickling prevented and globin imbalance improved	Few indels
Liao et al. 2023	Preclinical	Human HSPCs / β-thalassemia / HbE models	3–6 mice/replicates	ABE8e / ABE8e-SpRY	57.2–94.3	γ-globin increased from 1.6% to 20.3%; aberrant splicing reduced	Durable editing and improved globin balance	Few byproducts / noncoding off-targets

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

Study	Type	Disease / Model	n	Editing technique	Editing efficiency %	Main quantitative outcome	Clinical / functional response	Off-target / safety
						from 84.5% to 36.3%		

Ex-vivo CRISPR/Cas9-based gene editing has been playing out the potential of phasing out fundamental laboratory experimental research and enter clinical use in hemoglobinopathies, especially sickle cell disease and transfusion-dependent β-thalassemia. The best evidence is the work by Wang et al. [9], who reported the successful use of CRISPR-Cas9-edited autologous hematopoietic stem cells. These studies demonstrated that there was an increase in the production of fetal hemoglobin (HbF), the prevention or abolition of vaso-occlusive crisis in sickle cell disease, and other clinical benefits including the fact that transfusion is independent in most patients with 1 - thalassemia. Identifying the regulatory elements of genes involved in these processes (like the BCL11A enhancer) provides a molecular basis for restoration of fetal hemoglobin expression, to counter oxidative stress-perpetuated defective adult β-globin production.

And, perhaps, the power of this treatment lies in the fact that it uses the hematopoietic stem cells of the patient, and removes the need to find a matched donor, and many of the complications, which allogeneic stem cell transplantation causes. There exist a number of studies describing the BCL11A enhancer editing and the HBG1/HBG2 promoter editing (Sharma et al. [31] and Fu et al. [18]) which could support the idea that the most clinically advanced method currently is the reactivation of fetal hemoglobin. This is not so much like a repair of the mutation that causes a particular disease but rather more of a correction to the defect of the germline. Rather, it restores normal functioning by reinstating endogenous pathway of protective hemoglobin. Specifically, it applies to the case of sickle cell disease and β-thalassemia since different mutations cause the problem, but the induction of fetal hemoglobin can be used to alleviate both conditions.

Preclinical testing also provides necessary assistance in further development. The survival, engraftment, and maintenance of corrected hematopoietic stem cells in experimental models demonstrated by Lattanzi et al. [7] and Uchida et al. [39] are important to the long-term therapeutic success. The results suggest that long-term engraftment may become a possibility with the use of edited CD34+ cells; nonetheless, this will depend on the ability to optimize target editing efficiencies, stem cell fitness, and long-term hematopoietic remodelling to translate to clinical.

Two of the future generations of strategies that immediately succeed mutagenesis are base editing and prime editing. This was introduced by Newby et al. [10] and Zeng et al. [12] as a precision gene-editing technology, which has the advantage of correcting or modifying disease-related sequences but does not actually produce any breaks in the strands of the DNA molecules [8]. This could assist to mitigate the hazards of chromosomal rearrangements, massive deletions, and undesirable repair effects [37]. Analzalone et al. Reviews Reviews by Anzalone et al. Similarly, Chen and Liu [38] note that prime editing may have greater application potential in areas where very precise manipulation of the genome is required, although its clinical use in hemoglobinopathies

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

is currently in its infancy. Therefore, base and prime editing might be safer or more mutation-biased platforms as they progress through discovery, although CRISPR-Cas9 nuclease editing is currently the most clinically advanced platform.

Despite these innovations, safety continues to be a major issue. It is also required to do long-term follow-up to exclude off-target editing, non-specific genomic changes, clonal expansion, and the possibility of malignancy. The study of Cancellieri et al. [3] revealed that bioinformatics analysis (e.g., CRISPRitz) can be helpful in predicting the off-target location and add to a more secure evaluation prior to clinical implementation. Moreover, Goyal et al. [21] and Liggett et al. [22] identified the necessity of monitoring and future surveys in order to evaluate the clonal hematopoiesis and the possibility of genotoxicity after gene-based therapies. These concerns have strong implications on the implications of the edited hematopoietic stem cells reintroduced into the patient after a transplant, since this high number of cells may linger on the patient long after the transplant.

Overall, the reviewed studies indicate that ex vivo CRISPR-based therapy is a promising therapeutic approach to the management of hemoglobinopathies. Although there is an ever-growing body of evidence to support the clinical effectiveness of both BCL11A enhancer editing and fetal hemoglobin reactivation, base editing and prime editing may help in achieving even greater accuracy and safety in the future. Long-term safety data, cost-effectiveness, access to special treatment centers and de-escalation of conditioning-related toxicity will be the key factors in broadening clinical adoption.

Conclusion

Lastly, ex vivo CRISPR/Cas9 gene editing is a promising curative treatment of hereditary diseases such as sickle cell disease and β-thalassemia, which targets the genetic and regulatory mechanisms underlying the pathogenesis of the disease. The best clinical approach is BCL11A enhancer editing, which re-expresses fetal hemoglobin and results in positive clinical outcomes. Base editing, and prime editing, on the other hand, could provide safer and more precise alternatives in the future. Nonetheless, the long-term monitoring of genotoxicity remains complicated by the concern about off-target effects, mosaic, efficient delivery, and such issues as clonal hematopoiesis. Therefore, the therapy based on CRISPR could be proclaimed as a breakthrough, yet more long-term studies are required before the therapy could become accessible to many.

Recommendations

Long-term follow-up should consider safety, durability and late adverse events. To conclude, these studies have developed efficient methods of editing but one future concept is to refine these methods of delivering, in order to increase efficiency whilst maintaining the viability of stem cells. Base editing and prime editing are less risky approaches to precision-editing that need to be explored further. Off-target identification and screening of genomic safety should be conducted in a standardized manner before being applied clinically. This necessitates attention to the minimization of costs of treatment and the increase of access in the high-burden regions.

References

1. Papizan JB, Porter SN, Sharma A, Pruett-Miller SM. Therapeutic gene editing strategies using CRISPR-Cas9 for the β-hemoglobinopathies. J Biomed Res. 2020;35(2):115.

2. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. Nat Biotechnol. 2020;38(7):824-844.

3. Cancellieri S, Canver MC, Bombieri N, Giugno R, Pinello L, Pietrelli A. CRISPRitz: rapid, high-throughput and variant-aware in

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

silico off-target site identification for CRISPR genome editing. Bioinformatics. 2020;36(7):2001-2008.
4. Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med. 2021;384(3):252-260.
5. Frangoul H, Locatelli F, Sharma A, Bhatia M, Mapara MY, Molinari L, et al. Exagamglogene autotemcel for severe sickle cell disease. N Engl J Med. 2024;390(18):1649-1662.
6. Hirakawa MP, Krishnakumar R, Timlin JA, Carney JP, Butler KS. Gene editing and CRISPR in the clinic: current and future perspectives. Biosci Rep. 2020;40(4): BSR20200127.
7. Lattanzi A, Meneghini V, Pavani G, Amor F, Ramadier S, Felix T, et al. Development of β-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. Sci Transl Med. 2021;13(598): eabf2444.
8. Liao J, Karnik R, Gu H, Ziller MJ, Clement K, Tsankov AM, et al. Therapeutic adenine base editing of human hematopoietic stem cells. Nat Commun. 2023; 14:796.
9. Locatelli F, Lang P, Wall D, Mapara MY, Bhatia M, Walters MC, Frangoul H. Exagamglogene autotemcel for transfusion-dependent β-thalassemia. N Engl J Med. 2024;390(18):1663-1676.
10. Newby GA, Yen JS, Woodard KJ, Mayuranathan T, Lazzarotto CR, Li Y, et al. Base editing of haematopoietic stem cells rescues sickle cell disease in mice. Nature. 2021;595(7866):295-302.
11. Richter MF, Zhao KT, Eton E, Lapinaite A, Newby GA, Thuronyi BW, et al. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. Nat Biotechnol. 2020;38(7):883-891.
12. Zeng J, Wu Y, Ren C, Bonanno J, Shen AH, Shea D, et al. Therapeutic base editing of human hematopoietic stem cells. Nat Med. 2020;26(4):535-541.
13. Huang P, Pesiak CA, Ren R, Khandros E, Qin K, Keller CA, et al. HIC2 controls developmental hemoglobin switching by repressing BCL11A transcription. Nat Genet. 2022; 54:1417-1426.
14. Cabriolu A, Odek A, Zamparo L, Yuan H, Leslie CD, Sadelain M. Globin vector regulatory elements are active in early hematopoietic progenitor cells. Mol Ther. 2022; 30:2199-2210.
15. Magrin E, Semeraro M, Hebert N, Joseph L, Magnani A, Chalumeau A, et al. Long-term outcomes of lentiviral therapy for the β-hemoglobinopathies: the HGB-205 trial. Nat Med. 2022; 28:81-88.
16. Kanter J, Walters MC, Krishnamurti L, Mapara MY, Kwiatkowski JL, et al. Biologic and clinical efficacy of LentiGlobin for sickle cell disease. N Engl J Med. 2022; 386:617-628.
17. Locatelli F, Thompson AA, Kwiatkowski J, Porter JB, Thrasher AJ, et al. Betibeglogene autotemcel gene therapy for β-thalassemia. N Engl J Med. 2022; 386:415-427.
18. Fu B, Liao J, Chen S, Li W, Wang Q, Hu J, et al. CRISPR-Cas9-mediated gene editing of the BCL11A enhancer. Nat Med. 2022; 28:1573-1580.
19. Santorone S, Angelini S, Natale A, Vaddinelli D, et al. Survival and late effects of hematopoietic transplantation in thalassemia. Bone Marrow Transplant. 2022; 57:1689-1697.
20. Boulad F, Maggio A, Wang X, Moi P, et al. Lentiviral globin gene therapy in β-thalassemia. Nat Med. 2022; 28:63-70.
21. Goyal S, Tisdale J, Schmidt M, et al. Acute myeloid leukemia after gene therapy. N Engl J Med. 2022; 386:138-147.
22. Liggett LA, Cato LD, Weinstock JS, et al. Clonal hematopoiesis in sickle cell disease. J Clin Invest. 2022;132: e156060.
23. Solomou EE, Delaporta P, Stamatia L, et al. Clonal hematopoiesis in thalassemia. Blood. 2022; 140:8213-8214.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

24. Janik E, Niemcewicz M, Ceremuga M, Krzowski L, Saluk-Bijak J, Bijak M. Various aspects of a gene editing system—CRISPR-Cas9. Int J Mol Sci. 2020;21.
25. Walton RT, Christie KA, Whittaker MN, Kleinstiver BP. Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science. 2020; 368:290-296.
26. Kavanagh P, Fasipe T, Wun T. Sickle cell disease: a review. JAMA. 2022;328.
27. Darbari D, Sheehan V, Ballas S. The vaso-occlusive pain crisis in sickle cell disease: definition, pathophysiology, and management. Eur J Haematol. 2020;105.
28. Lugthart S, Ginete C, Kuona P, Brito M, Inusa B. An update review of new therapies in sickle cell disease: the prospects for drug combinations. Expert Opin Pharmacother. 2024.
29. Steinberg M. Fetal hemoglobin in sickle cell anemia. Blood. 2020;136.
30. Weber L, Frati G, Felix T, Hardouin G, Casini A, Wollenschlaeger C, et al. Editing a γ-globin repressor binding site restores fetal hemoglobin synthesis and corrects the sickle cell disease phenotype. Sci Adv. 2020;6.
31. Sharma A, Boelens JJ, Cancio M, Hankins JS, Bhad P, Azizy M, et al. CRISPR-Cas9 editing of the HBG1 and HBG2 promoters to treat sickle cell disease. N Engl J Med. 2023; 389:820-832.
32. Taher A, Musallam K, Cappellini M. β-Thalassemias. N Engl J Med. 2021;384.
33. Mitchell E, Spencer Chapman M, Williams N, Dawson KJ, Mende N, Calderbank EF, et al. Clonal dynamics of haematopoiesis across the human lifespan. Nature. 2022; 606:343-350.
34. Pipe SW, Gonen-Yaacovi G, Segurado OG. Hemophilia A gene therapy: current and next-generation approaches. Expert Opin Biol Ther. 2022.
35. Mancuso ME, Mahlangu JN, Pipe SW. The changing treatment landscape in haemophilia: from standard half-life clotting factor concentrates to gene editing. Lancet. 2021; 397:630-640.
36. Lara-Navarro IJ, Jaloma-Cruz AR. Current therapies in hemophilia: from plasma-derived factor modalities to CRISPR/Cas alternatives. Tohoku J Exp Med. 2022; 256:197-197.
37. Zhang X, Zhu B, Chen L, Xie L, Yu W, Wang Y, et al. Dual base editor catalyzes both cytosine and adenine base conversions in human cells. Nat Biotechnol. 2020; 38:856-860.
38. Chen P, Liu D. Prime editing for precise and highly versatile genome manipulation. Nat Rev Genet. 2023;24.
39. Uchida N, Drysdale CM, Nassehi T, Gamer J, Yapundich M, Bonifacino AC, et al. Preclinical evaluation for engraftment of CD34+ cells gene edited at the sickle cell disease locus in xenograft mice and non-human primates. Nat Commun. 2021; 12:3293.
40. Qin T, Chen Y, Chen G, Wu X, Xu J, Wang H, et al. Cas9-AAV6 gene correction of beta-globin in autologous SCD mouse model. Nat Commun. 2021; 12:686.
41. Zeng J, Chen H, Wu Y, et al. Advances in base editing technologies for hemoglobinopathies. Hum Genet. 2023;142(4):469-482.
42. de Lima IJEA, Rosa EMDSN, da Silva Neto GJ, Magalhães GG, Bordignon SS, Soares NP. CRISPR-Cas9 therapy in sickle cell anemia and β-thalassemia: clinical and preclinical evidence in a systematic review. REVISA. 2026;15(Esp 4):61-67.
43. Gaspar AB, Gaspar HB. Advances in gene therapy for inherited haemoglobinopathies. Hematol Rep. 2025;18(1):4.
44. Park SH, Karsenty C, Prasca-Chamorro D, Moyo B, Yoo B, Bao G. Gene-editing-based approaches for curing sickle cell disease. In: Engineering Approaches to Sickle Cell Disease: Innovations, Applications and Future Prospects. Cham: Springer Nature Switzerland; 2026. p. 167-203.
45. Jin Y, Zhang Y, Ling Y, Fu Q, He C. CRISPR/Cas9 in red blood cell-related diseases:

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 98 - 113

breakthroughs in gene editing and therapy. Blood Genomics. 2025.
46. Sun B. Application and breakthrough of CRISPR-Cas9 gene editing technology in the clinical treatment of sickle cell disease. MedScien. 2025;1(1).
47. Ahmed R, Alghamdi WN, Alharbi FR, Alatawi HD, Alenezi KM, Alanazi TF, et al. CRISPR/Cas9 system as a promising therapy in thalassemia and sickle cell disease: a systematic review of clinical trials. Mol Biotechnol. 2026;68(1):23-32.
48. Komal, Kaur P, Arora N, Sawale JA, Singh A. Transformative CRISPR-Cas9 technologies: a review of molecular mechanisms, precision editing techniques, and clinical progress in sickle cell disease. Curr Drug Metab. 2025.
49. Kansal R. Curing sickle cell disease by allogeneic hematopoietic stem cell transplantation toward in vivo HSC gene therapy. Genes. 2025;16(11):1367.
50. Butt H, Sathish S, London E, Le A, Li Q, Gudmundsdottir B, et al. Comparative analysis of CRISPR-Cas9, lentiviral transduction, and base editing for sickle cell disease in a murine model. Blood Adv. 2025.