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Review Article

CRISPR-CAS9-MEDIATED EX VIVO GENE EDITING FOR INHERITED HEMATOLOGICAL DISORDERS: ADVANCEMENTS, CHALLENGES, AND CLINICAL POTENTIAL

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ABSTRACT

Global healthcare systems have a great challenge in the form of inherited hematological diseases, which necessitates the development of new remedial strategies. By precisely targeting inherited abnormalities, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated protein 9 (Cas9)-mediated ex vivo gene editing has surfaced as a promising approach to treat these diseases. This review offers a comprehensive examination of the advancements, challenges, and clinical eventuality of CRISPR-Cas9-intermediated ex vivo gene editing for inherited hematological diseases. With advancements in CRISPR-Cas9 technology, the eventuality to correct inheritable mutations responsible for inherited hematological diseases is within reach. However, challenges such as off-target effects, immune responses, and ethical considerations need to be addressed for the safe and effective perpetration of this technology. A promising understanding of how CRISPR-Cas9-intermediated gene editing functions in practice is handed by ongoing clinical studies, giving rise to the possibility of advanced remedial approaches and bettered patient issues. By addressing these complications in a human-readable format, this review attempts to provide greater understanding and appreciation for the eventuality of CRISPR-Cas9 technology in revolutionizing the treatment landscape for these challenging disorders and contribute to the ongoing discussion in the field and facilitate further exploration towards effective treatments for these challenging disorders.

Keywords: CRISPR-Cas9, Gene editing, Inherited hematological disorders, Precision medicine, Ex vivo interventions, Genetic mutations, Clinical trials, Off-target effects, Immune responses, Therapeutic advancements

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INTRODUCTION

In the realm of healthcare, inherited hematological conditions, like sickle cell disease and beta-thalassemia, pose significant health complications globally due to inheritable abnormalities affecting hemoglobin production [1, 2]. An aberrant hemoglobin pattern, which gives red blood cells their distinctive sickle shape and makes them stiff, is reflective of sickle cell diseases. This condition increases the risk of infections, organ damage, and painful occurrences [3]. Conversely, beta-thalassemia results in inadequate erythropoiesis, anemia, and complications such as bone deformities and organ damage due to diminished or nonexistent beta-globin chain synthesis. Traditional treatments focus on symptom management and supportive care, such as blood transfusions and medications, but do not address the underlying genetic defects, necessitating lifelong management [4–6].

Amidst the limitations of traditional treatments, Clustered regularly interspaced short palindromic repeats(CRISPR)-CRISPR-associated protein 9 (Cas9) technology, derived from a bacterial immune system has surfaced as a promising frontier for addressing inherited blood diseases like sickle cell disease and beta-thalassemia [5–8]. Its precision in editing DNA sequences offers the potential to correct genetic mutations underlying these conditions, shifting treatment focus from symptom management to addressing the root cause. Understanding the genetic mechanisms driving these disorders, from mutations in the beta-globin gene causing abnormal hemoglobin in sickle cell disease to disruptions in hemoglobin production due to mutations in the Hemoglobin Subunit Beta (HBB) gene in beta-thalassemia, is crucial for guiding targeted interventions [9, 10].

At the forefront of gene editing technology, CRISPR-Cas9 offers unequaled precision, efficiency, and scalability, making it ideally suited for ex vivo gene editing operations in inherited blood disorders. This approach involves editing patients' Hematopoietic Stem Cells (HSCs) outside the body before reintroduction, potentially offering long-term therapeutic benefits and even cures [11–13]. In this review, we delve into the advancements, challenges, and clinical potential of CRISPR-Cas9-mediated ex vivo gene editing for inherited hematological disorders. Our objectives include providing a comprehensive overview of CRISPR-Cas9 technology, examining recent advances in gene editing strategies, and evaluating efficacy and safety through preclinical and ongoing clinical trials. By addressing challenges and limitations, we aim to enhance understanding and pave the way for future innovations in precision medicine, ultimately offering restorative cures and hope for patients' brighter, healthier futures [14-16].

Advancements in crispr-Cas9 technology

Evolution of CRISPR-Cas9: from discovery to application

The journey of CRISPR-Cas9 technology, originating from bacterial vulnerable systems like Streptococcus pyogenes, has been marked by significant mileposts [17]. The first major advance in the evolution of CRISPR-Cas9 gene editing came with the adaption of the system for use in eukaryotic cells, enabling precise targeting of DNA sequences in mammalian cells and revolutionizing genetic research [18]. Subsequent advancements, such as the engineering of high-fidelity Cas9 variants and the introduction of smaller Cas9 orthologs and alternative CRISPR systems like CRISPR from *Prevotella and Francisella* 1 (CPF1), have expanded the gene-editing toolkit, enhanced precision and effectiveness while minimizing off-target effects [19-21].

Molecular mechanisms of CRISPR-Cas9 editing

At the heart of CRISPR-Cas9 technology lies a significant molecular tool capable of precisely targeting and modifying specific regions of the genome [22, 23]. It consists of two main factors the Cas9 protein and a guide RNA (gRNA). The Cas9 protein acts as a molecular scissor, cutting double-stranded DNA at specific target sequences guided by the gRNA through base pairing. Initially, the gRNA recognizes and binds to a specific DNA sequence adjacent to a Protospacer Adjacent Motif (PAM) sequence. Once bound, Cas9 induces a Double-Stranded Break (DSB) in the DNA, triggering the cell's natural DNA repair mechanisms such as Non-homologous End Joining (NHEJ) and Homology-Directed Repair (HDR) [24–26].

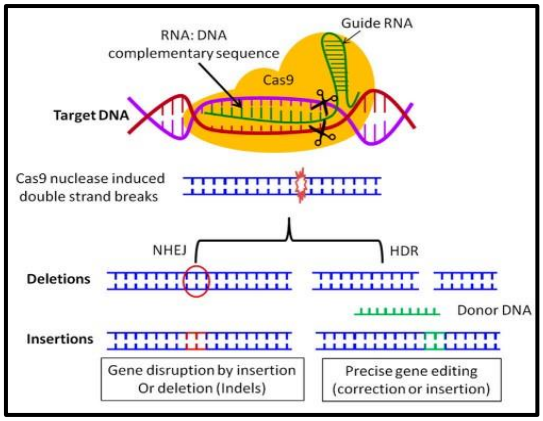

Fig. 1: Schematic diagram illustrating the molecular mechanism of CRISPR-Cas9-mediated gene editing, including guide RNA (gRNA) targeting, Cas9 nuclease activity, and DNA repair mechanisms. (Source: Abdelnour SA, Xie L, Hassanin AA, Zuo E, Lu Y. The potential of CRISPR/Cas9 gene editing as a treatment strategy for inherited diseases. Vol. 9, frontiers in cell and developmental biology. Frontiers media S. A.; 2021) [27]

The DNA repair process following Cas9-mediated cleavage can lead to the introduction of desired genetic modifications, such as gene knockouts, insertions, or replacements. By precisely targeting specific DNA sequences, CRISPR-Cas9 enables researchers to manipulate the genome with unprecedented delicacy and effectiveness [28, 29].

Recent Innovations in CRISPR-Cas9 editing techniques

In the fast-paced world of genetic engineering, recent advancements have expanded the capabilities of CRISPR-Cas9 for precise genome editing. One significant advancement is the development of base editing and prime editing techniques, which enable targeted nucleotide substitutions or precise insertions and deletions without double-stranded breaks, reducing off-target effects [30–32]. Improvements in CRISPR-Cas9 specificity include high-fidelity Cas9 variants and modified gRNAs, enhancing safety and efficacy. Additionally, novel approaches, such as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), enable precise regulation of gene expression without altering the underlying DNA sequence [33]. One of the pivotal challenges in employing the full eventuality of CRISPR-Cas9 for remedial purposes lies in delivering the editing ministry to target cells effectively [34, 35]. This has led to the development of various delivery systems, including viral vectors such as Adeno-Associated Viruses (AAVs) and lentiviruses, known for efficiently transducing various cell types, including hematopoietic stem cells. Non-viral delivery methods, such as lipid nanoparticles and electroporation, offer alternative strategies for delivering CRISPR-Cas9 factors, potentially reducing the risk of immune responses and off-target effects [36–39]. Optimization of delivery systems aims to enhance the clinical feasibility of CRISPR-Cas9-mediated ex vivo gene editing for inherited hematological diseases. Continuous innovation and enhancement have driven the evolution of CRISPR-Cas9 technology, leading it to evolve from an introductory exploration tool to a promising therapeutic approach [40, 41].

CRISPR Cas9 ex vivo gene editing strategies for inherited hematological disorders

Inherited hematological disorders encompass a diverse spectrum of genetic conditions affecting the production, structure, or function of blood cells, often resulting in significant morbidity and mortality. Examples include sickle cell disease, thalassemia, hemophilia, and various types of anemia. These diseases are generally caused by mutations in genes involved in hematopoiesis, hemoglobin synthesis, or coagulation pathways. Manifestations can range from mild to severe, with symptoms similar as chronic anemia, pain crises, organ damage, and increased vulnerability to infections or bleeding [42, 43].

Table 1: Summary of genetic mutations associated with inherited blood disorders

S. No.	Inherited blood disorder	Affected gene	Type of mutation	Phenotype	Reference
1.	Sickle Cell Disease	HBB	Missense	Hemolytic Anemia, Vaso-Occlusive Crises, Beta-Thalassemia	[44]
2.	Beta-Thalassemia	HBB	Nonsense	Microcytic anemia, hepatosplenomegaly	[12]
3.	Hemophilia A	F8	Insertion/Deletion	Prolonged bleeding, joint deformities	[44]
4.	Hemophilia B	F9	Missense	Prolonged bleeding, spontaneous hemorrhage	[44]
5.	Fanconi Anemia	FANCA	Nonsense	Bone marrow failure, developmental abnormalities	[9]
6.	G6PD Deficiency	G6PD	Deletion	Hemolytic anemia, jaundice	[45]
7.	Alpha-Thalassemia	HBA	Deletion	Hemolytic anemia, skeletal abnormalities	[33]
8.	Thrombocytopenia	MPL	Frameshift	Low Platelet Count, Easy Bruising	[46]
9.	Thalassemia Intermedia	HBB	Insertion	Anemia, Fatigue	[47]

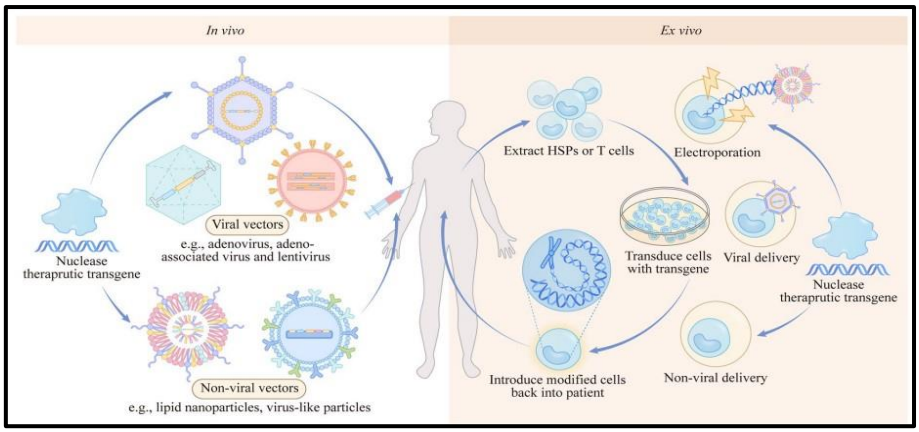

Fig. 2: EX vivo vs. *in vivo* strategies for therapeutic CRISPR genome editing (Source: Li R, Wang Q, She K, Lu F, Yang Y. CRISPR/Cas systems usher in a new era of disease treatment and diagnosis. Vol. 3, Molecular Biomedicine. (Springer; 2022) [54]

Inherited blood diseases pose significant challenges for affected individuals and their families, challenging lifelong operation and care. Advances in inheritable testing, prenatal screening, and treatment modalities, including gene remedy and HSCs transplantation, offer hope for improved outcomes. CRISPR-Cas9 technology holds a significant pledge for the treatment of inherited hematological diseases through precise gene editing [48–50]. In the environment of these diseases, similar to sickle cell complaint and beta-thalassemia, CRISPR-Cas9 can be utilized in various ways to correct underlying genetic mutations. One operation involves *in vivo* gene editing, where CRISPR-Cas9 is directly delivered into the patient's body to target specific cells or tissues affected by the disorder. Another approach is ex vivo gene editing, which entails modifying hematopoietic stem cells or T cells outside the body before reintroduction [51, 52]. While ex vivo strategies offer greater control over editing effectiveness and particularity, *in vivo* approaches offer the advantage of simplicity and eventuality broader applicability [44, 53].

With a focus on modifying HSCs, ex vivo gene editing, which leverages the potential of CRISPR-Cas9 technology, presents a possible treatment option for hereditary anemia disorders. These HSCs, obtained from the patient's peripheral blood or bone marrow, are targeted due to their ability to generate all blood cell types. CRISPR-Cas9 enables precise modification of specific genomic areas within these HSCs, allowing for the correction of underlying genetic mutations responsible for conditions like sickle cell disease and beta-thalassemia [55-57]. Frequently, mutations in particular genes essential for hematopoiesis and hemoglobin synthesis result in inherited hematological diseases. The HBB gene, encoding the beta-globin subunit of hemoglobin, is a primary target for ex vivo gene editing in conditions like sickle cell disease and beta-thalassemia, where mutations lead to abnormal hemoglobin structure or reduced production [46, 47]. To achieve successful editing of the HBB gene, various strategies are employed. These could involve the insertion of therapeutic transgenes, the removal of disease-causing regions, or the use of CRISPR-Cas9-mediated homology-directed repair. By targeting these key genes and customized editing techniques, scientists aim to restore normal hematopoiesis and alleviate symptoms. With the precision of CRISPR-Cas9 technology, ex vivo gene editing holds promise for individualized and effective treatments for these challenging diseases, offering hope for improved outcomes and a higher quality of life for patients worldwide [58, 59].

Clinical potential and therapeutics applications

Preclinical studies serve as pivotal stepping monuments in assessing the eventuality of CRISPR-Cas9-intermediated gene editing for inherited hematological diseases, such as sickle cell disease and beta-thalassemia. In animal models, such as mice and non-human primates, experimenters have successfully employed CRISPR-Cas9 to precisely target and edit defective genes, restoring normal hematological parameters and alleviating disease symptoms [60]. A significant preclinical study showed that CRISPR-Cas9 could potentially fix the sickle cell mutation in a mouse model of sickle cell disease. In this work, researchers effectively edited the appropriate genetic sequence in hematopoietic stem cells using CRISPR-Cas9, leading to the creation of functional red blood cells with normal hemoglobin levels. These edited cells were then transplanted back into the mice, leading to a significant reduction in sickling and enhancement in overall health. Similarly, in a rat model of beta-thalassemia, CRISPR-Cas9-mediated gene editing increased normal hemoglobin synthesis and reduced symptoms [61, 62]. Another noteworthy preclinical work focused on the successful delivery of CRISPR-Cas9 components into hematopoietic stem cells derived from non-human primates, leading to the production of healthy blood cells and bone marrow replenishment [63, 64]. These preclinical studies emphasize the versatility and efficacity of CRISPR-Cas9-intermediated gene editing across different animal models, pressing its eventuality as a transformative remedial strategy for inherited hematological diseases. Moreover, preclinical studies have played a pivotal part in relating and addressing challenges similar to off-target effects, immune responses, and the need for optimized delivery styles to ensure effective and precise editing, which must be addressed before advancing to clinical trials [65–67].

Clinical trials represent a critical stage in the journey of CRISPR-Cas9 gene editing technology from the laboratory to real-world operations for inherited hematological diseases. These trials aim to estimate the safety, efficacity, and feasibility of CRISPR-Cas9 interventions in mortal cases, furnishing precious perceptivity into their eventuality as remedial strategies [68, 69]. Ongoing and completed clinical trials for conditions like sickle cell disease and beta-thalassemia validate promising preclinical results and restate them into tangible clinical issues. Initial outcomes from these trials have shown varying degrees of success, with some reporting advancements in hematological parameters, reduction of disease-related symptoms, and dragged ages of disease remission in treated patients [70]. For illustration, recent trials for sickle cell disease have demonstrated increased levels of normal hemoglobin and reduced complications in patients treated with CRISPR-Cas9-edited hematopoietic stem cells, offering hope for its potential as a transformative remedy [71, 72].

Table 3: Summary of clinical trials utilizing CRISPR-Cas9 mediated ex vivo gene editing for inherited blood disorders

Clinical trial ID	Phase	Disease	Treatment protocol	Outcome	Current status	References
NCT04925206	Phase 1	Beta-Thalassemia	Infusion of CRISPR-Edited CD34+Hematopoietic Stem Cells	Increased Hemoglobin Levels, Reduced Transfusion Dependence	Recruiting	[73]
NCT04205435	Phase 1/2	Beta-thalassemia, Sickle Cell Disease	Transplantation of CRISPR-Edited Hematopoietic Stem Cells	Improved Hemoglobin Levels, Reduced Disease Complications	Active not recruiting	[74]
NCT04774536	Phase 1/2	Sickle Cell Disease	Transplantation of CRISPR-Edited Hematopoietic Stem Cells	Improved Hemoglobin Levels, Reduced Disease Symptoms	Ongoing	[73]
NCT04293185	Phase 3	Sickle Cell Disease	Transplantation of CRISPR-Edited Hematopoietic Stem Cells	Increased Hemoglobin Levels, Reduced Transfusion Dependence	Recruiting	[62]
NCT04592458	Phase 1	Beta-Thalassemia	Transplantation of CRISPR-Edited CD34+Hematopoietic Stem Cells	Increased Hemoglobin Levels, Reduced Transfusion Dependence	Ongoing	[62]
NCT03728322	Phase 1	Beta-Thalassemia	Transplantation of CRISPR-Edited Hematopoietic Stem Cells	Increased Hemoglobin Levels, Reduced Disease Symptoms	Recruiting	[75]
NCT03745287	Phase 1	Sickle Cell Disease, Beta-Thalassemia	Transplantation of CRISPR-Edited CD34+Hematopoietic Stem Cells	Improved Hemoglobin Levels, Reduced Disease Symptoms	Recruiting	[76]
NCT03655678	Phase 1	Beta-Thalassemia	Transplantation of CRISPR-Edited CD34+Hematopoietic Stem Cells	Improved Hemoglobin Levels, Reduced Transfusion Dependence	Recruiting	[77]

Despite these promising results, clinical trials using CRISPR-Cas9 for inherited hematological disorders face several challenges, including optimizing delivery methods to target HSCs effectively while mitigating off-target effects and addressing immune responses to edited cells [78–80]. A further obstacle that needs to be overcome is the ethical and regulatory issues that surround the application of gene

editing technology in clinical settings. Nonetheless, ongoing trials demonstrate CRISPR-Cas9's potential as a treatment avenue. Looking forward, tailored strategies based on patient genetic profiles hold promise for enhancing therapeutic outcomes and minimizing risks associated with CRISPR-Cas9 interventions [81, 82].

Challenges and limitations

Off-target effects

Understanding the complexities of CRISPR-Cas9-mediated ex vivo gene editing for inherited blood diseases requires addressing significant challenges, such as off-target effects. These effects involve unexpected genetic alterations occurring at sites other than the intended target, potentially disrupting essential genes or activating oncogenes [45, 76, 83]. Despite CRISPR-Cas9's precision, the risk of off-target changes remains, necessitating strategies like protein engineering, precise gRNA design, and bioinformatics tools to mitigate these effects before clinical application. Optimizing delivery methods further enhances targeting accuracy, ensuring the safety and effectiveness of ex vivo gene editing techniques [84–87].

Immunogenicity and host responses

Immunogenicity and Host Responses pose another challenge in CRISPR-Cas9-mediated gene editing, particularly concerning the eventuality of immune responses triggered by the insertion of edited cells into the patient's body [73]. Inserting edited cells using CRISPR-Cas9 may trigger immune responses, leading to cell rejection or inflammation. Addressing immunogenicity involves selecting immunocompatible editing reagents, modulating immune responses through immunosuppressive remedies, and engineering edited cells to evade immune detection. Additionally, advances in gene editing technologies, such as the development of non-viral delivery systems, may help reduce immunological responses and improve the tolerability of ex vivo gene editing interventions [88, 89].

Ethical and regulatory consideration

Exploring ethical considerations surrounding CRISPR-Cas9 gene editing is essential for navigating the complex geography of genetic medicine. Concerns related to germline editing raise profound ethical questions about the eventuality of unintended consequences and the implications for future generations [90]. Equitable access to therapies, consent, privacy, and societal implications must be addressed. Regulatory frameworks must balance scientific innovation with human welfare and ethical principles, necessitating robust oversight and transparent governance structures to ensure responsible and ethical use of CRISPR-Cas9-mediated ex vivo gene editing for inherited hematological disorders [91, 92].

Future directions and opportunities

Looking ahead, the future of CRISPR-Cas9-mediated ex vivo gene editing for inherited hematological disorders is ripe with implicit potential, presenting a horizon of instigative openings and new avenues for exploration. Future endeavors may concentrate on enhancing the precision, efficiency, and safety of gene editing techniques, steering in a new era of targeted therapeutic interventions [93-95]. Furthermore, the integration of CRISPR-Cas9 with other cutting-edge technologies, such as artificial intelligence and machine learning, could enable more precise targeting of genetic mutations and substantiated treatment strategies acclimatized to individual patients. Additionally, solidarity with arising fields like regenerative medicine, synthetic biology, single-cell genomics, bioinformatics, and tissue engineering offers exciting opportunities to explore the complications of inherited hematological disorders at a position of granularity never before imagined. This interdisciplinary approach not only deepens our understanding of disease mechanisms but also illuminates novel therapeutic targets and strategies, offering renewed hope for patients and clinicians alike [96–100]. Likewise, as we navigate the path toward clinical restatement, it's imperative to address the challenges and ethical considerations that accompany the use of CRISPR-Cas9 in mortal cases. Robust regulatory frameworks and transparent communication are essential to ensure responsible use, prioritizing patient safety and autonomy [74, 101]. Exploring combination therapies and integrating gene editing with modalities like gene therapy or small molecule inhibitors offers a holistic approach to target multiple aspects of disease pathology. Additionally, refining disease models will accelerate preclinical research, advancing therapeutic development for inherited hematological disorders [75, 102, 103].

CONCLUSION

The journey of CRISPR-Cas9-intermediated ex vivo gene editing for inherited hematological diseases is both a testament to scientific invention and a reflection of the complications essential in biomedical exploration. Advancements in understanding, refining, and applying CRISPR-Cas9 technology have propelled the field forward, offering unknown openings to address inheritable diseases at their root cause. Preclinical studies have provided insights into efficacy and safety, while ongoing clinical trials offer glimpses of real-world impact. Despite challenges such as off-target effects and ethical considerations, the clinical potential of CRISPR-Cas9 remains promising. Ongoing trials show encouraging results, and as technology evolves and challenges are addressed, new possibilities emerge. Embracing emerging technologies and approaches promises to reshape treatment landscapes, offering hope to affected families. With unwavering commitment and collaboration, we stand poised to realize the clinical eventuality of CRISPR-Cas9, steering in a new era of hope and healing for patients around the globe.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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