Proceedings of the 5th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/96/2025.21435

Advancements in CRISPR Technologies and Treatment of Genetic Disorders

Youxi Chen[1,a,*]

[1]*University of Toronto, Toronto, Ontario, M5S 1A1, Canada*
a. youxi.c@outlook.com
**corresponding author*

Abstract: When CRISPR-Cas9, short for Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) with CRISPR-associated protein 9 (Cas9), was successfully harnessed for genome editing in the early 2010s, it marked a new era for biotechnology. The high precision, efficiency, and adaptability of CRISPR-Cas9 have unlocked extraordinary potential in medicine, agriculture, and industrial biology, underscored by the awarding of the Nobel Prize in Chemistry in 2020 to its pioneers. This paper reviews follow-on advancements to the technology addressing challenges, including off-target effects and inefficient delivery systems, and explores its transformative applications in treating genetic disorders, including sickle cell disease, transfusion-dependent β-thalassemia, and cystic fibrosis. Additionally, it highlights ongoing hurdles management of such as high costs and safety and efficacy of heritable gene editing. This study shows that addressing these challenges and fostering ethical and collaborative advancements will be essential for CRISPR technologies, which can fulfill their transformative potential in improving human life quality.

Keywords: CRISPR-Cas9, genetic disorders, genome editing, biotechnology, medicine

1. Introduction

In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9), along with their subsequent advancements (collectively referred to as CRISPR technologies), have played a pivotal role in research and applications within the fields of gene therapy, agriculture, and industrial biotechnology. By enabling targeted modifications of genetic material, CRISPR technologies have accelerated the development of therapies for previously untreatable genetic disorders, improved crop resilience, and enhanced the production of bio-based chemicals and fuels.

At its core, CRISPR-Cas9 employs a ribonucleoprotein complex—comprising a guide RNA (gRNA) and the Cas9 protein—to introduce site-specific double-strand DNA breaks (DSBs) [1]. The cell's intrinsic repair mechanisms, such as non-homologous end joining (NHEJ) or homology-directed repair (HDR), can then be harnessed to disrupt or precisely modify genes. However, the rapid expansion of its applications has exposed significant challenges, including off-target effects that can compromise safety and delivery inefficiencies that limit clinical efficacy.

In response, researchers have made substantial strides in refining CRISPR technologies. Innovations like prime editing (PE) have reduced off-target activity while enabling more precise genetic modifications without requiring DSBs. Simultaneously, advancements in delivery systems,

DOI: 10.54254/2753-8818/96/2025.21435

including viral and non-viral vectors and nanoparticles (NPs), are being employed to enable the safe and effective transport of CRISPR components into target cells. As CRISPR technologies advance, concerns such as the high costs of treatment and ethical concerns have arised, requiring further action and collaborative efforts among stakeholders to ensure equitable access and responsible use of these groundbreaking tools.

This study employes a literature review methodology to explore the advancements in CRISPR technologies and their transformative potential in treating genetic disorders and highlight challenges arising from rapid adoption of CRISPR technologies. By synthesizing these findings, the paper aims to provide a current view on the capabilities of CRISPR technologies while advocating for collaborative efforts to address lingering issues.

2. Efforts to Advance CRISPR-Cas9 Technology

While CRISPR-Cas9 offers remarkable advantages, its broader application has highlighted key technical challenges including off-target effects and inefficient delivery mechanisms. Continued efforts have focused on addressing these limitations for safe and effective clinical application of CRISPR technologies. This section highlights some key developments and their roles in overcoming critical barriers and advancing the field toward clinical and commercial viability.

2.1. Off-target Effects

One major limitation of CRISPR-Cas9 is the unintended genetic modifications it may introduce. Off-target effects refer to instances where CRISPR-Cas9 introduces DSBs at unintended genomic locations, leading to unwanted insertions or deletions (indels). For example, while HDR has been widely used to achieve precise DNA changes, it suffers from low efficiency and a high rate of unintended indels due to competing end-joining repair pathways. HDR also requires the presence of an external donor DNA template, which further complicates its use in most therapeutically relevant cell types.

To address these challenges, many alternative approaches were explored, with PE appearing as a major advancement. Developed in the lab of David R. Liu at the Broad Institute, PE introduces precise insertions and deletions without requiring DSBs or donor DNA templates [2]. This system directly writes new genetic information into a specific DNA site by utilizing a Cas9 endonuclease fused to a reverse transcriptase, guided by a PE guide RNA (pegRNA) that specifies target site and encodes the desired edit [2]. In human cells, PE achieved targeted modifications with efficiencies ranging from 20% to 50% depending on the locus and cell type [2]. The study also revealed significant reductions in off-target activity compared to Cas9 nucleases: off-target editing was detected at only 3 out of 16 known Cas9 off-target sites, and just 1 site exhibited an off-target editing efficiency exceeding 1% [2]. These findings underscore the potential of PE to address off-target issues associated with earlier genome editing technologies.

2.2. Delivery Mechanisms

An effective delivery mechanism is a critical factor for the successful therapeutic application of gene-editing technologies. CRISPR-Cas9's clinical potential heavily depends on the efficient and safe transfer of its components into target cells. To achieve this, both viral and non-viral vectors have been explored extensively.

Viral vectors are considered highly effective tools for gene transfer, capable of targeting specific cell types or tissues and being engineered to express therapeutic genes [3]. Key players such as adeno-associated viruses (AAV), adenoviruses and lentiviruses have been extensively utilized and optimized in clinical models and trials [4]. However, despite their effectiveness, the broader clinical adoption

DOI: 10.54254/2753-8818/96/2025.21435

of viral vectors is hindered by issues such as unwanted mutations, high off-target effects and safety concerns, such as the risk of triggering severe immune responses in patients [4-5].

To overcome these limitations, alternatives such as non-viral *ex vivo* delivery systems and NPs have been explored. Non-viral *ex vivo* delivery systems utilize non-viral vectors to modify the genome of specific cells *in vitro*. The edited cells are then transplanted back into the patient, where they induce a regenerative effect [4]. Recently, non-viral *ex vivo* gene-editing therapy was conducted for transfusion-dependent β-thalassemia (TDT), a genetic blood disorder characterized by severe anemia due to inadequate production of functional hemoglobin [6]. In a clinical study of 52 patients, the treatment achieved notable success, with neutrophil and platelet engraftment observed in all patients [6]. Among the 35 patients with sufficient follow-up data, 32 (91%) achieved transfusion independence ($P<0.001$) [6].

Where *in vivo* delivery is essential for effective gene therapy, NPs have emerged as promising alternatives to viral vectors to overcome the latter's limitations by offering more specific targeting and reduced stimulation of immune response [7]. Lipid-based NPs, in particular, have reduced immunogenicity and can be engineered or modified to enhance biodistribution, the dispersion of the vector from the site of administration, during systemic delivery. This flexibility is particularly valuable for delivering CRISPR tools, as it enables the treatment of a broader spectrum of diseases [8].

Advancements in addressing CRISPR-Cas9's key limitations, including off-target effects and delivery challenges, have been pivotal in moving the technology closer to clinical and therapeutic viability. PE has emerged as a significant breakthrough, offering precise and efficient gene editing while reducing the risk of unintended genetic modifications. In parallel, explorations of delivery mechanisms, ranging from optimized viral vectors to NPs, are expanding the feasibility of delivering CRISPR components safely and effectively to target cells. These developments demonstrate how overcoming technical barriers enhances both the precision and practicality of CRISPR applications.

3. Treating Genetic Disorders

Advancements of the CRISPR-Cas9 technology have driven a surge in preclinical research and clinical trials exploring gene-editing therapies for genetic disorders. This section highlights two key developments that showcase the ability of CRISPR-based techniques to provide durable and potentially curative treatments.

3.1. Sickle Cell Disease and Transfusion-dependent β-thalassemia

In December 2023, CRISPR Therapeutics and Vertex Pharmaceuticals received U.S. Food and Drug Administration (FDA) approval for Casgevy, the first CRISPR-based therapy, marking a milestone in gene-editing treatments [9]. Casgevy is a one-time therapy used to treat TDT and sickle cell disease (SCD), which is one of the most common genetic disorders worldwide that leads to the formation of abnormally shaped red blood cells that block blood flow and cause painful vaso-occlusive episodes [9]. By targeting the BCL11A erythroid-specific enhancer, a key regulator that suppresses fetal hemoglobin production, Casgevy effectively reactivates fetal hemoglobin, alleviating disease symptoms [10].

In clinical trials, Casgevy achieved approximately 80±6% allele modification rate at the target locus with no evidence of off-target editing [10]. Two patients—one with TDT and one with SCD—received the edited autologous hematopoietic stem and progenitor cells (HSPCs) after undergoing myeloablation. More than a year since the treatment (18 months for patient with TDT and 15 months for patient with SCD), both patients saw substantial increase in fetal hemoglobin level (patient with TDT saw levels of fetal hemoglobin increase from 0.3g per deciliter at baseline to 13.1g per deciliter

DOI: 10.54254/2753-8818/96/2025.21435

at month 18; patient with SCD saw fetal hemoglobin level increase from 9.1% at baseline to 43.2% at month 15) [10]. Importantly, the fetal hemoglobin was pancellular, indicating it was present in nearly all red blood cells. Further, both patients achieved transfusion independence, and the SCD patient experienced complete elimination of vaso-occlusive episodes, a hallmark complication of the disease [10].

These results highlight the potential of CRISPR-Cas9 gene editing to offer durable and potentially curative treatments for TDT and SCD by reactivating fetal hemoglobin production, mimicking the protective effects observed in individuals with hereditary persistence of fetal hemoglobin.

3.2. Cystic Fibrosis

Cystic fibrosis (CF), an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is another severe genetic disorder that may benefit from CRISPR-based gene editing. The most common mutation, F508del, results in the deletion of three nucleotides that encode phenylalanine 508 in the CFTR protein, leading to protein misfolding and degradation [11]. This prevents the CFTR protein from reaching the cell surface, compromising chloride ion transport and causing thick mucus buildup in organs such as the lungs and pancreas.

Although current treatments, including small-molecule modulators such as elexacaftor, tezacaftor, and ivacaftor, have substantially improved patient outcomes, these therapies require lifelong administration [12]. This ongoing need for treatment underscores the demand for durable therapeutic solutions, prompting researchers to explore CRISPR-based strategies.

In 2024, a study led by Sousa AA, Hemez C and colleagues from David R. Liu's lab - the same lab that developed PE in 2019 – highlighted that their results from using optimized PE for CFTR F508del correction could pave the path for a durable one-time treatment for CF. Initial attempts to correct the F508del mutation using the original PE system yielded average editing efficiencies of less than 0.5%, revealing the mutation's high resistance to correction by PE [11]. Through applying six optimizations on PE, correction efficiency increased to 58% in immortalized bronchial epithelial cells and 25% in primary airway epithelial cells derived from CF patients. The researchers found minimal off-target editing under the tested conditions [11].

Further, optimized PE restored CFTR channel function to over 50% of wild-type levels in primary airway epithelial cells derived from patients with CF, achieving results comparable to those of the combination of elexacaftor, tezacaftor, and ivacaftor treatments [11]. Additionally, the study demonstrated that optimized PE produced an edit-to-indel ratio 3.5 times higher than conventional HDR methods, indicating PE-induced efficiency and its potential to reduce off-target effects [11].

The advancements in CRISPR-based therapies, exemplified by the FDA-approved Casgevy for SCD and TDT, and the promising application of PE to treat CF, highlight the versatility of CRISPR in addressing a wide array of genetic conditions, paving the way for durable, effective treatments for diseases that were previously considered incurable.

4. Remaining Challenges

Despite significant advancements, challenges remain that must be addressed to ensure the broader adoption of CRISPR technologies for genetic disorders.

4.1. High Cost and Accessibility

Gene therapies' prohibitively high costs pose significant challenges for equitable access. The first approved CRISPR-based therapy Casgevy costs USD$2.2 million [13]. Similarly, other gene therapies are priced at comparably high levels. Zolgensma, approved for treating spinal muscular

DOI: 10.54254/2753-8818/96/2025.21435

atrophy in 2020, costs USD$2.1 million, while Lenmeldy, approved for treating metachromatic leukodystrophy in 2024, is currently the world's most expensive treatment, costing USD$4.25 million [14-15].

To date, the small number of patients treated has allowed large payers to absorb the aggregate costs, but the rapid increase in gene therapy approvals signals an impending challenge [16]. Over the past seven years, the number of FDA-approved single-dose gene therapies has risen from zero to 17, and this figure is projected to soar to 85 by 2032 [16]. This growth highlights the urgency of addressing affordability and access to these innovative, potentially life-changing treatments.

In response, collaborative efforts such as the recent white paper by Institute for Clinical and Economic Review and Tufts Medical Center aim to provide policy and market-based solutions [16]. The paper emphasizes that unlocking the full potential of gene therapies will require a concerted effort from all stakeholders—payers, policymakers, and industry leaders—to develop practical and economically sustainable pricing and payment strategies [16]. Achieving equitable access to these transformative treatments while preserving the healthcare system's sustainability demands a comprehensive approach that combines innovative pricing models, risk-sharing agreements, and sustainable reimbursement frameworks, supported by key policy reforms [16].

4.2. Germline Editing and Ethical Concerns

The immense therapeutic potential of CRISPR has led researchers to explore a wide range of applications, including its use in embryos to investigate the potential for eliminating disease-causing mutations from the human germline. However, in November 2018, Chinese scientist He Jiankui announced the birth of twin girls whose embryonic genomes were edited using CRISPR-Cas9 to disable a pathway used by HIV to infect cells, it marked a highly controversial milestone in genome editing, as it was the first known instance of gene editing applied to human embryos for reproductive purposes [17]. The announcement raised significant concerns, as the potential off-target effects and long-term consequences of such editing remained unknown, sparking a global debate on the ethical and responsible use of genome-editing technologies.

In response, the World Health Organization (WHO) established an Expert Advisory Committee in December 2018 and released a governance framework in 2021 to call for human genome editing to be conducted safely, effectively, and ethically [18].

Discussions surrounding the permissibility of heritable genome editing are ongoing. After the Third International Summit on Human Genome Editing in 2023, the consensus was that heritable genome editing lacks sufficient preclinical evidence to confirm its safety and efficacy, and societal discussions and policy debates remain unresolved [19]. It was concluded that heritable genome editing should only be considered if it meets rigorous standards for safety and efficacy, is legally authorized, and is governed by a robust oversight system. At present, these prerequisites have not been fulfilled.

While germline genome editing holds significant potential to prevent serious genetic diseases and improve human health, it remains fraught with complex ethical and societal challenges. Until robust preclinical evidence, stringent regulatory frameworks, and broad societal consensus are achieved, the application of germline genome editing is likely to remain prohibited. These ongoing debates reflect the delicate balance between advancing scientific innovation and ensuring ethical responsibility in the pursuit of human genome editing.

5. Conclusion

CRISPR technologies, with advancements like prime editing and explorations of various delivery mechanisms, are expanding the scope of potential treatments for complex genetic disorders.

DOI: 10.54254/2753-8818/96/2025.21435

Promising results from CRISPR-based therapies, exemplified by FDA's approval of Casgevy and outcome from applying prime editing for diseases like CF, underscores the transformative potential of this technology in offering durable and potentially curative solutions.

Despite these achievements, challenges remain in ensuring the equitable and safe adoption of CRISPR technologies. High costs of treatment and ethical concerns surrounding heritable genome editing highlight the need for ongoing collaborative efforts among researchers, policymakers, and industry leaders. While this paper outlines published opinion on criteria that must be met for heritable genome editing, it leaves room for further exploration of the rigorous standards needed to ensure safety and efficacy. With sustained research, innovation, and ethical stewardship, CRISPR technologies hold the potential to deliver life-changing solutions to a broad population, shaping a future where genetic disorders and other global challenges are met with groundbreaking improvement in human life quality.

References

[1] *Doudna, J. A., & Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science (New York, N.Y.), 346(6213), 1258096. https://doi.org/10.1126/science.1258096*

[2] *Anzalone, A.V., Randolph, P.B., Davis, J.R., et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576, 149–157.*

[3] *Warnock, J.N., Daigre, C., Al-Rubeai, M. (2011). Introduction to Viral Vectors. In: Merten, OW., Al-Rubeai, M. (eds) Viral Vectors for Gene Therapy. Methods in Molecular Biology. Humana Press, 737, 1-25.*

[4] *Khoshandam, M., Soltaninejad, H., Mousazadeh, M., Hamidieh, A. A., & Hosseinkhani, S. (2023). Clinical applications of the CRISPR/Cas9 genome-editing system: Delivery options and challenges in precision medicine. Genes & diseases, 11(1), 268–282.*

[5] *Rouatbi, N., McGlynn, T., & Al-Jamal, K. T. (2022). Pre-clinical non-viral vectors exploited for in vivo CRISPR/Cas9 gene editing: an overview. Biomaterials Science, 10(13), 3410–3432.*

[6] *Locatelli, F., Lang, P., et al. (2024). Exagamglogene Autotemcel for Transfusion-Dependent β-Thalassemia. The New England journal of medicine, 390(18), 1663–1676.*

[7] *Chen, F., Alphonse, M., & Liu, Q. (2020). Strategies for nonviral nanoparticle-based delivery of CRISPR/Cas9 therapeutics. Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology, 12(3), e1609.*

[8] *Glass, Z., Li, Y. & Xu, Q. (2017). Nanoparticles for CRISPR–Cas9 delivery. Nat Biomed Eng, 1, 854–855.*

[9] *Dimitrievska, M., Bansal, D., et al. (2024). Revolutionising healing: Gene Editing's breakthrough against sickle cell disease. Blood reviews, 65, 101185.*

[10] *Frangoul, H., Altshuler, D., et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. The New England journal of medicine, 384(3), 252–260.*

[11] *Sousa, A.A., Hemez, C., Lei, L. et al. (2025). Systematic optimization of prime editing for the efficient functional correction of CFTR F508del in human airway epithelial cells. Nat. Biomed. Eng. 9, 7–21.*

[12] *Allessandra DiCorato. (2024). Prime editing efficiently corrects cystic fibrosis mutation in human lung cells. Broadinstitute. https://www.broadinstitute.org/news/prime-editing-efficiently-corrects-cystic-fibrosis-mutation-human-lung-cells#*

[13] *Sheridan, C. (2023). The world's first CRISPR therapy is approved: who will receive it? Nature Biotechnology, 42(1), 3–4.*

[14] *Rigter, T., Klein, D., Weinreich, S. S., & Cornel, M. C. (2021). Moving somatic gene editing to the clinic: routes to market access and reimbursement in Europe. European journal of human genetics : EJHG, 29(10), 1477–1484.*

[15] *Bansal, A. (2024). Lenmeldy becomes world's most expensive drug. Pharmaceutical Technology. April, 2.*

[16] *Phares, S., Trusheim, M., Emond, S. K., Pearson, S. D., Institute for Clinical and Economic Review, & NEWDIGS, Tufts Center for Biomedical System Design. (2024). Managing the Challenges of paying for gene therapy: Strategies for market action and policy reform. In White Paper: Managing the Challenges of Paying for Gene Therapy [Report]. https://icer.org/wp-content/uploads/2024/04/Managing-the-Challenges-of-Paying-for-Gene-Therapy-_-ICER-NEWDIGS-White-Paper-2024_final.pdf*

[17] *Cyranoski, D., & Ledford, H. (2018). Genome-edited baby claim provokes international outcry. Nature, 563(7733), 607–608.*

[18] *World Health Organization. (2021). WHO Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing. Human Genome Editing: recommendations. Geneva.. Licence: CC BY-NC-SA 3.0 IGO*

DOI: 10.54254/2753-8818/96/2025.21435

[19] *National Academies of Sciences, Engineering, and Medicine. (2023). Third International Summit on Human Genome Editing: Expanding Capabilities, Participation, and Access: Proceedings of a Workshop—in Brief. Washington, DC: The National Academies Press. https://doi.org/10.17226/27066*