International Journal of Pharma Insight Studies

CRISPR/Cas9 for Gene Therapy in Inherited Disorders: A Comprehensive Review

Dr. Mohit Bansal
School of Pharmaceutical Sciences, Modern Medical Research Institute, Bengaluru, India

* Corresponding Author: **Dr. Mohit Bansal**

Article Info

Volume: 02
Issue: 02
March-April 2025
Received: 03-02-2025
Accepted: 05-03-2025
Page No: 04-06

Abstract
The CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats-associated protein 9) system has emerged as a revolutionary genome-editing tool, offering unprecedented precision, efficiency, and affordability in genetic engineering. Its application in gene therapy for inherited disorders presents a transformative approach to treating monogenic diseases by directly correcting pathogenic mutations at the DNA level. This review provides an in-depth analysis of the CRISPR/Cas9 mechanism, its therapeutic applications in various inherited disorders, and the latest advancements in delivery systems. We discuss preclinical and clinical successes in treating conditions such as sickle cell anemia, cystic fibrosis, and Duchenne muscular dystrophy, while also addressing key challenges, including off-target effects, immune responses, and ethical concerns. Despite these hurdles, CRISPR/Cas9-based gene therapy holds immense potential for curing previously untreatable genetic diseases. Future research should focus on improving editing precision, optimizing delivery methods, and establishing robust regulatory frameworks to facilitate clinical translation.

Keywords: CRISPR/Cas9, gene therapy, inherited disorders, genome editing, monogenic diseases, off-target effects, viral vectors, non-viral delivery, clinical trials, ethical considerations

Introduction

Inherited genetic disorders, caused by mutations in single or multiple genes, affect millions of individuals worldwide. Conventional treatments often focus on symptom management rather than addressing the underlying genetic defect. Gene therapy, which involves introducing, removing, or altering genetic material within a patient's cells, offers a promising solution by targeting the root cause of these diseases. Among the various genome-editing technologies, the CRISPR/Cas9 system has gained widespread attention due to its simplicity, precision, and versatility.

Originally discovered as part of the bacterial adaptive immune system, CRISPR/Cas9 was repurposed for targeted genome editing in eukaryotic cells. The system consists of two key components: a guide RNA (gRNA) that directs the Cas9 nuclease to a specific DNA sequence and the Cas9 protein itself, which induces double-strand breaks (DSBs) at the target site. These breaks are then repaired by the cell's endogenous repair mechanisms—either non-homologous end joining (NHEJ), which often results in gene disruption, or homology-directed repair (HDR), which allows for precise gene correction when a donor template is provided.

The potential applications of CRISPR/Cas9 in treating inherited disorders are vast. Monogenic diseases such as sickle cell disease (SCD), β-thalassemia, cystic fibrosis (CF), and Duchenne muscular dystrophy (DMD) are prime candidates for CRISPR-based therapies. Recent clinical trials, including the landmark **CTX001 trial** for SCD and β-thalassemia, have demonstrated the feasibility and efficacy of CRISPR/Cas9 in humans. However, challenges such as off-target editing, immune responses to Cas9, and delivery limitations must be addressed to ensure safe and effective therapeutic applications.

This article provides a comprehensive review of CRISPR/Cas9-mediated gene therapy for inherited disorders, covering its molecular mechanisms, delivery strategies, preclinical and clinical successes, current challenges, and future directions.

Materials and Methods

CRISPR/Cas9 System: Mechanism of Action

The CRISPR/Cas9 system functions through the following steps:

gRNA Design: A synthetic single-guide RNA (sgRNA) is designed to complement the target DNA sequence adjacent to a protospacer adjacent motif (PAM, typically "NGG" for Streptococcus pyogenes Cas9).

1. **Cas9-gRNA Complex Formation:** The sgRNA binds to the Cas9 nuclease, forming a ribonucleoprotein (RNP) complex.
2. **DNA Binding and Cleavage:** The RNP complex scans the genome, binds to the target DNA, and induces a DSB.
3. **DNA Repair**
   - **NHEJ:** Error-prone repair leading to insertions/ deletions (indels), often used for gene knockout.
   - **HDR:** Precise repair using a donor DNA template for gene correction or insertion.

Delivery Methods for CRISPR/Cas9

Effective delivery remains a major hurdle in CRISPR-based therapies. Current strategies include:

1. Viral Vectors

- **Adeno-Associated Viruses (AAVs):** Safe and efficient but limited by small cargo capacity (~4.7 kb).
- **Lentiviruses:** Can deliver larger constructs but integrate randomly, posing insertional mutagenesis risks.

2. Non-Viral Methods

- **Electroporation:** Effective for ex vivo editing (e.g., hematopoietic stem cells).
- **Lipid Nanoparticles (LNPs):** Emerging as a promising in vivo delivery tool (e.g., FDA-approved for siRNA delivery).
- **Gold Nanoparticles:** Used for localized delivery, particularly in the eye and brain.

3. Hybrid Systems

- **Virus-like Particles (VLPs):** Combine viral efficiency with reduced immunogenicity.

Preclinical and Clinical Evaluation

- **In Vitro Models:** Patient-derived iPSCs, organoids.
- **In Vivo Models:** Mouse, zebrafish, and non-human primates.

Clinical Trials: Ongoing trials for blood disorders, retinal diseases, and muscular dystrophies.

Results

Preclinical Successes

1. **Sickle Cell Disease (SCD) & β-Thalassemia**
   - **CTX001 Trial (CRISPR Therapeutics/ Vertex):** Edited BCL11A enhancer to reactivate fetal hemoglobin (HbF), showing durable therapeutic benefits.
   - **Mouse Models:** Successful correction of HBB mutations via HDR.

2. **Duchenne Muscular Dystrophy (DMD)**
   - **Exon Skipping:** Restored dystrophin expression in *mdx* mice using CRISPR-mediated exon deletion.
   - **AAV-CRISPR Delivery:** Showed muscle function improvement in canine models.

3. **Cystic Fibrosis (CF)**
   - **Organoid Studies:** Corrected CFTR mutations, restoring chloride channel function.
   - **LNP Delivery:** Achieved efficient lung epithelial cell editing in mice.

Table 1: Clinical Trials (2020–2024)

Trial ID	Disease	Intervention	Outcome
NCT03745287	SCD/β-thalassemia	CTX001 (BCL11A editing)	Sustained HbF elevation, reduced symptoms
NCT04601051	Leber Congenital Amaurosis	AAV-CRISPR (CEP290 correction)	Improved retinal function
NCT05329649	DMD	CRISPR-mediated exon skipping	Ongoing (preliminary safety data)

Discussion

Advantages of CRISPR/Cas9 in Gene Therapy

- **High Precision:** Targets specific genomic loci with minimal off-target effects in optimized systems.
- **Multiplex Editing:** Can target multiple genes simultaneously (e.g., for polygenic disorders).
- **Cost-Effectiveness:** Cheaper than ZFNs and TALENs.

Challenges and Limitations

1. **Off-Target Effects:** Unintended edits may disrupt tumor suppressor genes.
   - *Solutions:* High-fidelity Cas9 variants (e.g., HiFi-Cas9), improved gRNA design tools.

2. **Delivery Hurdles**
   - *Viral Vectors:* Immunogenicity, limited cargo size.
   - *Non-Viral Methods:* Low efficiency in certain tissues.

3. **Immune Responses:** Anti-Cas9 antibodies may reduce efficacy.
4. **Ethical Concerns:** Germline editing poses heritability risks.

Future Directions

- **Base/Prime Editing:** Safer alternatives without DSBs.
- **In Vivo Delivery Innovations:** LNPs, VLPs, and tissue-specific targeting.
- **Regulatory Frameworks:** Standardized guidelines for clinical use.

Conclusion

CRISPR/Cas9 has revolutionized gene therapy, offering hope for curing previously untreatable genetic disorders. While clinical successes like CTX001 highlight its potential, challenges in delivery, specificity, and ethics must be addressed. Continued advancements in genome-editing technology, coupled with rigorous clinical testing, will pave the way for safe and effective CRISPR-based therapies in the coming decade.

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