

Review Article

CRISPR-Based Gene Editing in Clinical Therapeutics: Challenges and Breakthroughs

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How to cite this article:

Manasa M S. CRISPR-Based Gene Editing in Clinical Therapeutics: Challenges and Breakthroughs. *Asian. J. Herb. Med. Phytochem. Res.* 2025; 1(1): 5-10.

Date of Submission: 2025-03-21

Date of Acceptance: 2025-05-12

A B S T R A C T

CRISPR-Cas systems have rapidly transformed the landscape of gene editing, offering unprecedented precision and efficiency in modifying the genome. Over the past decade, CRISPR technology has evolved from a molecular biology tool into a promising platform for clinical therapeutics, with applications spanning monogenic disorders, cancer, infectious diseases, and beyond. This review provides a comprehensive overview of the current state of CRISPR-based therapies in clinical development, highlighting key breakthroughs such as ex vivo edited T cells for cancer immunotherapy and in vivo editing for conditions like transthyretin amyloidosis and sickle cell disease. Despite these advances, significant challenges remain, including delivery efficiency, off-target effects, immunogenicity, and ethical considerations. We discuss innovative strategies to overcome these hurdles, including base and prime editing, novel delivery vectors, and transient CRISPR systems. Additionally, regulatory, manufacturing, and access-related aspects of CRISPR therapeutics are examined. As the field moves closer to widespread clinical application, understanding both the scientific and translational barriers is critical to harnessing the full potential of gene editing in medicine.

Keywords: CRISPR-Cas9, Gene Editing, Clinical Therapeutics, Genome Engineering, Base Editing, Prime Editing, In Vivo Delivery, Ex Vivo Therapy, Off-Target Effects, Genetic Diseases, Immunogenicity, Precision Medicine

Introduction

The advent of CRISPR-Cas systems has marked a paradigm shift in the field of genome engineering, offering a precise, programmable, and accessible method for targeted genetic modification. Originally discovered as an adaptive immune mechanism in bacteria, the CRISPR-Cas9 system has been repurposed into a versatile molecular tool capable of inducing site-specific double-stranded breaks in DNA, enabling gene knockouts, insertions, or corrections with relative ease. Since its first demonstration in mammalian cells in 2013, CRISPR has rapidly advanced from laboratory

research into the clinic, spurring a wave of innovation in therapeutic development.

Gene editing holds particular promise for treating a wide array of genetic diseases that have limited or no effective therapies. Early clinical trials have already demonstrated encouraging results for monogenic disorders such as sickle cell disease, β -thalassemia, and Leber congenital amaurosis. Moreover, CRISPR-based strategies are being actively explored in oncology, infectious diseases (e.g., HIV), and immunotherapy, offering new hope for conditions previously deemed untreatable.

Despite these promising developments, several critical challenges must be addressed before CRISPR-based therapies can achieve widespread clinical adoption. These include efficient and safe delivery to target cells or tissues, minimizing off-target effects, managing immune responses to CRISPR components, and navigating complex ethical and regulatory frameworks. Additionally, as newer iterations of CRISPR—such as base editing, prime editing, and CRISPR-associated transposases—emerge, their potential to overcome current limitations and expand therapeutic applications continues to grow.

This review aims to provide a comprehensive overview of CRISPR-based gene editing in clinical therapeutics, focusing on major breakthroughs, ongoing clinical trials, and key technical and translational challenges. By examining the current landscape and future directions, we aim to offer insights into how CRISPR technology is reshaping the future of precision medicine.

Mechanism of CRISPR-Cas Systems

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems, originally discovered as part of the adaptive immune system in bacteria and archaea, provide defense against invading nucleic acids such as phages and plasmids. These systems are broadly classified into two classes (Class 1 and Class 2), with Class 2 systems (particularly CRISPR-Cas9) being the most widely used in genome editing due to their simplicity and effectiveness.¹

Natural Function

The natural CRISPR-Cas immune response proceeds through three main stages:

Adaptation (Spacer Acquisition)

- When a bacterium is infected by a virus, short fragments of the invader's DNA (called "protospacers") are captured and integrated into the CRISPR array in the host genome.
- These DNA sequences are flanked by repeat sequences and become "spacers" that serve as a molecular memory.

Expression and Processing

- The CRISPR locus is transcribed into a long precursor RNA (pre-crRNA), which is processed into short CRISPR RNAs (crRNAs).
- In Cas9 systems, a trans-activating crRNA (tracrRNA) is also required to form a crRNA-tracrRNA duplex that guides the Cas protein.

Interference

- The mature crRNA-tracrRNA complex guides the Cas protein (e.g., Cas9) to a complementary DNA sequence in the invading genome.

- Cas9 scans the DNA for a specific Protospacer Adjacent Motif (PAM, e.g., NGG for SpCas9)
- Upon PAM recognition and crRNA-target hybridization, Cas9 induces a double-stranded break (DSB) at the target site.

Engineered CRISPR-Cas9 for Gene Editing

In biotechnology, this natural system has been simplified and repurposed:

- The crRNA and tracrRNA are combined into a single synthetic guide RNA (sgRNA) that directs Cas9 to a specific genomic locus.
- Cas9 can be delivered into cells as DNA, RNA, or protein along with the sgRNA.

DNA Repair After Cas9 Cutting

Following a Cas9-induced double-strand break, the cell attempts to repair the damage using one of two primary mechanisms:

Non-Homologous End Joining (NHEJ)

- Error-prone repair that often introduces insertions or deletions (indels), leading to gene disruption or knockouts.

Homology-Directed Repair (HDR)

- A precise repair mechanism that uses a homologous DNA template to incorporate specific changes—useful for gene correction or insertion.
- HDR is less efficient and typically active only in dividing cells.

Variants and Enhancements

- **Base Editors:** Use catalytically impaired Cas proteins fused to enzymes (like deaminases) to convert single bases without cutting DNA.
- **Prime Editors:** Use a reverse transcriptase fused to Cas9 nickase and a prime editing guide RNA (pegRNA) to perform small insertions, deletions, or base changes without requiring DSBs.
- **Cas12 and Cas13 Systems:** Target DNA and RNA respectively, expanding the range of therapeutic applications.

Clinical Breakthroughs

Hematological Disorders

- **Sickle Cell Disease and β -Thalassemia:** The most advanced CRISPR clinical applications involve editing hematopoietic stem cells to reactivate fetal hemoglobin production. CTX001 (developed by CRISPR Therapeutics and Vertex Pharmaceuticals) has shown promising results in early-phase trials.²

Cancer Immunotherapy

- CRISPR has been employed to knock out PD-1 or TCR genes in T-cells, enhancing immune responses against tumors. Early-phase trials have demonstrated feasibility and safety.

Genetic Blindness

- Trials targeting CEP290 mutations in Leber Congenital Amaurosis (LCA10) via CRISPR-based in vivo editing (e.g., Editas Medicine's EDIT-101) mark the first human application of in vivo CRISPR in the eye.

Challenges in Clinical Translation

Despite the rapid progress and transformative potential of CRISPR in medicine, several critical challenges must be overcome to ensure safe, effective, and scalable clinical use:

Delivery Efficiency and Specificity

Challenge

- Efficient and tissue-specific delivery of CRISPR components (Cas protein and guide RNA) remains a major bottleneck.

Issues

- Viral vectors (e.g., AAV, lentivirus): Limited packaging capacity, risk of insertional mutagenesis, and immunogenicity.
- Non-viral methods (e.g., lipid nanoparticles, electroporation): May have low efficiency or be limited to ex vivo settings.

Need

- Targeted, transient, and non-immunogenic delivery systems, especially for in vivo applications.

Off-Target Effect

Challenge

- Unintended DNA cleavage at sites similar to the target sequence can cause mutations and genomic instability.

Solutions Under Investigation

- High-fidelity Cas9 variants (e.g., eSpCas9, SpCas9-HF1).
- Improved guide RNA design and specificity screening.
- Transient expression systems to reduce exposure time.

Immunogenicity

Challenge

- The immune system may recognize Cas proteins (derived from bacterial species) as foreign, leading to immune responses that reduce efficacy or cause adverse effects.

Concerns

- Pre-existing immunity to Cas9 (especially from *Streptococcus pyogenes*).
- Inflammatory responses to delivery vectors (especially AAV).

Approaches

- Use of Cas orthologs from less common species.
- Immune suppression or transient expression of CRISPR components.

Control of Editing Outcomes

Challenge

- Variable efficiency and unpredictability of repair outcomes (e.g., with NHEJ vs HDR).

HDR Limitations

- HDR is inefficient in non-dividing cells.
- Often outcompeted by NHEJ.

Emerging Solutions

- Prime editing and base editing for precision edits without DSBs.
- Use of small molecules or timing strategies to bias repair pathways.

Scalability and Manufacturing

Challenge

- GMP-grade production of Cas proteins, guide RNAs, and delivery systems for clinical use is complex and costly.

Issues

- Batch consistency.
- Regulatory compliance.
- Long lead times for personalized therapies (e.g., ex vivo modified cells).

Ethical and Regulatory Considerations

Challenge

- Ethical concerns, particularly around germline editing, and evolving regulatory frameworks.

Key Points

- Somatic cell editing is generally more accepted, but long-term safety is still uncertain.
- Regulatory agencies (FDA, EMA) are developing new standards for evaluating gene editing therapies.
- Public perception and trust are critical to clinical acceptance.

Durability and Long-Term Safety

Challenge

- Long-term outcomes of gene editing are still unknown, especially for in vivo edits.

Concerns

- Oncogenic transformation due to off-target effects.
- Persistence of edits and expression in target tissues.

Approach:

- Long-term monitoring in clinical trials.
- Use of self-limiting systems (e.g., transient mRNA delivery).

Emerging Innovations in CRISPR-Based Clinical Therapeutics

As CRISPR technologies continue to mature, a wave of next-generation innovations is emerging to enhance safety, precision, and applicability in clinical settings. These innovations aim to address key technical and translational challenges while opening up new therapeutic frontiers.³

Base Editing: Precision Without Double-Strand Breaks

Description

- Base editors enable single-nucleotide conversions (e.g., C→T or A→G) without creating double-stranded DNA breaks.

Advantages

- Lower risk of off-target indels.
- High efficiency in both dividing and non-dividing cells.

Clinical Potential

- Treatment of point mutations in genetic diseases (e.g., sickle cell anemia, progeria).

Key Tools

- BE3 (cytidine base editor), ABE (adenine base editor).

Prime Editing: Versatile and Precise Genome Editing

Description

A “search-and-replace” system that fuses a Cas9 nickase with reverse transcriptase, guided by a prime editing guide RNA (pegRNA).

Advantages

- Capable of all 12 types of base substitutions, insertions, and deletions.
- No need for donor DNA or DSBs.

Applications

- Correcting complex mutations in rare genetic disorders.

CRISPR-Cas12 and Cas13 Systems: Beyond Cas9

Cas12

- Targets DNA like Cas9 but creates staggered cuts, useful in diagnostics and potential gene editing.

Cas13

- Targets RNA, allowing transient and reversible editing.

Therapeutic Use

- RNA editing for diseases like ALS, and viral RNA targeting (e.g., SARS-CoV-2, influenza).

In Vivo Delivery Innovations

Lipid Nanoparticles (LNPs)

- Used successfully in mRNA vaccines and now adapted for CRISPR delivery (e.g., in Intellia’s NTLA-2001 trial for transthyretin amyloidosis).

Engineered Viral Vectors

- AAV variants with improved tropism and reduced immunogenicity.

Virus-Free Systems

- Electroporation, cell-penetrating peptides, and exosome-based methods are under development for safer delivery.⁴

Epigenome Editing and CRISPRi/CRISPRa

- CRISPRi (interference) and CRISPRa (activation) use dead Cas9 (dCas9) fused to transcriptional repressors or activators to regulate gene expression without altering DNA.
- Applications: Modulating gene networks in cancer, neurological disorders, and autoimmunity.

Self-Destruct and Inducible CRISPR Systems

- **Description:** Engineered Cas9 systems that are conditionally activated (e.g., by light, small molecules, or tissue-specific promoters).
- **Benefit:** Enhanced temporal and spatial control, reducing off-target and systemic effects.

Synthetic Biology and Programmable Circuits

- Synthetic gene circuits using CRISPR components allow programmable control of gene expression, useful for responsive drug delivery and smart cell therapies (e.g., logic-gated CAR-T cells).

Next-Generation Cell Therapies

CRISPR-edited T cells

- Knockout of immune checkpoints (e.g., PD-1), insertion of synthetic receptors, or enhancement of persistence.

Stem Cell Therapies

- Autologous iPSCs edited ex vivo for diseases like sickle cell disease, retinal degeneration.

Multiplexed and High-Throughput Editing

- **Description:** Simultaneous editing of multiple genomic loci using pooled sgRNA libraries or multiplex delivery systems.
- **Applications:** Modeling complex diseases, engineering polygenic traits, or synthetic biology applications.

CRISPR Diagnostics (CRISPR-Dx)

- Use of Cas12 and Cas13 in diagnostics, enabling highly sensitive, portable detection of nucleic acids (e.g., SHERLOCK, DETECTR).
- **Relevance:** Companion diagnostics in personalized medicine and infectious disease screening.

Future Directions

The continued evolution of CRISPR-based technologies is poised to reshape the landscape of clinical therapeutics, offering curative potential for a wide range of genetic and acquired diseases. However, realizing this promise requires addressing existing limitations while expanding the frontiers of what is biologically and technically possible. Several key areas will define the trajectory of CRISPR-based clinical innovation in the coming decade:⁵

Improved Delivery Systems for In Vivo Applications Need

Develop safer, more efficient, and tissue-specific delivery platforms.

Focus Areas

- Next-generation non-viral vectors (e.g., lipid nanoparticles, engineered extracellular vesicles).
- Biodegradable delivery platforms that reduce systemic exposure.
- Organ-targeted delivery using cell-specific ligands or RNA aptamers.

Expansion Beyond Rare Genetic Diseases

Current Limitation

- Most clinical trials target rare monogenic disorders.

Next Frontier

- Application in complex polygenic diseases (e.g., diabetes, cardiovascular disorders).
- Integration with multi-omics data to guide polygenic risk modification strategies.
- Expansion into aging-related conditions, neurodegeneration, and autoimmune diseases.

Advanced Gene Regulation Technologies

Emerging Tools

CRISPR interference (CRISPRi), activation (CRISPRa), and epigenome editing.

Future Application

- Fine-tuning gene networks rather than permanent gene alteration.
- Potentially reversible and more ethically acceptable alternatives for long-term therapy.

Enhanced Precision and Control

Technological Focus

- Continued refinement of base and prime editors.
- Development of condition-responsive CRISPR systems (e.g., small molecule-, light-, or temperature-inducible).
- Introduction of programmable gene circuits for responsive editing in vivo.

Scalable and Accessible Manufacturing

Challenge

- High cost and logistical complexity of personalized CRISPR therapies.

Solutions

- Automation and modular platforms for GMP manufacturing.
- Development of off-the-shelf gene therapies with universal donor cells or in vivo editing.
- Cost-reduction strategies for broader access in low- and middle-income countries (LMICs).

Ethical, Regulatory, and Social Considerations

Anticipated Developments

- Establishment of global ethical frameworks for somatic and germline editing.
- Policies for equitable access, consent in pediatric use, and long-term surveillance.
- Public engagement to build trust and literacy around gene editing.

Long-Term Monitoring and Safety Infrastructure

Requirement

Comprehensive, multi-year follow-up protocols for CRISPR-treated patients.

Focus

- Integration of longitudinal genomic monitoring with clinical records.
- Use of digital biomarkers and AI analytics to predict adverse outcomes.
- Establishment of global CRISPR registries for safety signal detection.

Synergy with Emerging Technologies

Integration Opportunities

- Artificial intelligence for guide RNA design, target selection, and patient stratification.
- Synthetic biology for programmable cell therapies.
- RNA therapeutics and nanomedicine to complement CRISPR platforms.

Conclusion

CRISPR-based gene editing holds immense promise in revolutionizing clinical therapeutics. While early clinical trials are encouraging, the full realization of CRISPR's potential hinges on overcoming technical, ethical, and regulatory challenges. Continued innovation and collaboration across disciplines are essential to translate CRISPR from a powerful tool into a mainstream medical therapy.

Conflict of Interest: None

Source of Funding: None

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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