Global Research Journal in Engineering, Science, Technology and Management (GRJESTM)
Volume 01, Issue 05 (September-October) 2025, pp. 208-218
GRJESTM :: An odaswa™ Journal
ISSN : 3107-3611 (Online)

REVIEW ARTICLE

CRISPR-Cas Systems in Targeted Gene Therapy: Advances, Challenges, and Clinical Applications

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Abstract. CRISPR-Cas systems have emerged as a revolutionary genome-editing platform, enabling precise and efficient modifications of DNA sequences in living organisms. The simplicity, versatility, and programmability of CRISPR-Cas, particularly the Cas9 endonuclease, have accelerated research in targeted gene therapy for inherited disorders, cancer, and viral infections. This review highlights recent advancements in CRISPR-based therapeutic strategies, including ex vivo and in vivo genome editing, delivery mechanisms, and off-target minimization techniques. Key challenges such as immunogenicity, delivery efficiency, ethical considerations, and regulatory barriers are discussed. Clinical applications of CRISPR-Cas therapies in hemoglobinopathies, metabolic disorders, and cancer immunotherapy are evaluated, with emphasis on ongoing clinical trials and translational potential. Emerging technologies, including base editing, prime editing, and epigenetic modulation, further expand the therapeutic possibilities of CRISPR systems. Despite technical and ethical hurdles, CRISPR-Cas-mediated gene therapy holds transformative potential for personalized medicine, offering long-term cures for previously untreatable diseases.

Keywords: Green building, sustainable practices, environmental impact, building efficiency, transaction costs, budgeting errors

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1. Introduction

Gene therapy has emerged as a promising strategy to correct or modify defective genes responsible for a wide spectrum of inherited and acquired diseases. By directly targeting the underlying genetic cause, gene therapy offers the potential for curative rather than symptomatic treatment, distinguishing it from conventional pharmacological approaches [1,2]. Early gene therapy strategies predominantly relied on viral vector-mediated gene addition, where therapeutic genes were delivered using retroviral, adenoviral, or adeno-associated viral vectors. While such approaches demonstrated initial success, their clinical application has been limited by challenges such as insertional mutagenesis, unpredictable transgene expression, immunogenic responses, and off-target effects [3,4].

The development of CRISPR-Cas systems has revolutionized the field of genome engineering by providing a precise, efficient, and programmable platform for targeted DNA modification [5]. Derived from bacterial adaptive immune mechanisms, CRISPR-Cas systems recognize and cleave foreign genetic elements via RNA-guided endonucleases, which has been harnessed for controlled genome editing in mammalian cells [6]. Among various CRISPR effectors, Cas9 has become the most widely adopted due to its simplicity, high efficiency, and robust targeting capability. Guided by a synthetic single-guide RNA (sgRNA), Cas9 introduces site-specific double-strand breaks (DSBs) at the genomic locus of interest, which are subsequently repaired by the host cell through non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways [7,8]. This enables precise gene disruption, correction, or insertion, depending on the repair mechanism and design strategy.

CRISPR-Cas9 has been successfully applied across diverse therapeutic contexts, including the correction of monogenic disorders, functional genomics studies, cancer immunotherapy, and antiviral therapies [9,10]. Its advantages over earlier genome-editing methods, such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), include simpler design, higher specificity, multiplexing capability, and potential for permanent genome modification [11,12]. Recent technological advances such as base editing and prime editing further enhance the precision of CRISPR-mediated modifications by allowing single-nucleotide changes or targeted insertions/deletions without generating DSBs, thereby reducing unwanted mutations and improving safety profiles [13,14].

The remarkable flexibility and efficacy of CRISPR-Cas systems have accelerated translational research, leading to ongoing clinical trials targeting diseases such as hemoglobinopathies, inherited retinal disorders, and refractory cancers [15,16]. Despite these advances, several challenges remain, including off-target effects, immune responses, efficient in vivo delivery, and ethical considerations, which must be addressed before widespread clinical adoption. This paper provides a comprehensive review of CRISPR-Cas-mediated gene therapy, highlighting recent technological innovations, clinical applications, and the challenges that need to be overcome to fully realize its therapeutic potential.

2. CRISPR-Cas Systems and Mechanisms

CRISPR-Cas systems are naturally occurring adaptive immune mechanisms in bacteria and archaea, designed to recognize and neutralize invading genetic elements such as phages and plasmids [17]. These systems have been adapted for precise genome editing in eukaryotic cells due to their programmability, efficiency, and ease of design. CRISPR-Cas systems are broadly classified into two major classes based on the composition of their effector complexes and mechanism of target recognition [7,18]:

- Class 1: Multi-subunit effector complexes that require several Cas proteins to form a functional interference complex. This class includes Type I, Type III, and Type

IV systems. These systems are relatively more complex and are less commonly used in therapeutic genome editing.

- Class 2: Single-protein effectors that function independently as the nuclease component, simplifying their application for genome engineering. This class includes Type II (Cas9), Type V (Cas12), and Type VI (Cas13) systems. These systems have become the preferred tools for targeted gene therapy due to their simplicity and high efficiency [19].

Among these, Cas9 (Type II) is the most widely employed for therapeutic genome editing. Cas9 is guided by a single-guide RNA (sgRNA) that directs the nuclease to a specific DNA sequence complementary to the sgRNA. Target recognition requires the presence of a protospacer adjacent motif (PAM) immediately downstream of the target sequence. Upon binding, Cas9 introduces a site-specific double-strand break (DSB) in the DNA [20]. The repair of this DSB by cellular pathways determines the final genome modification:

- Non-homologous end joining (NHEJ): A rapid but error-prone repair mechanism that may introduce small insertions or deletions (indels), often used to disrupt gene function.
- Homology-directed repair (HDR): A precise repair pathway that can introduce specific nucleotide changes or insertions when a donor template is provided, enabling gene correction or replacement [21].

To improve editing precision and minimize undesired mutations, advanced CRISPR technologies such as base editors and prime editors have been developed. Base editors combine a catalytically impaired Cas protein with a deaminase enzyme to directly convert one nucleotide to another without generating a DSB, reducing the risk of off-target indels [22]. Prime editors, on the other hand, utilize a fusion of Cas9 nickase and reverse transcriptase, guided by a prime editing guide RNA (pegRNA), to introduce targeted insertions, deletions, or base conversions with high accuracy [6,23]. These innovations enhance the therapeutic potential of CRISPR systems by enabling precise, predictable, and safer genome modifications.

3. Advances in Targeted Gene Therapy

The advent of CRISPR-Cas systems has significantly advanced the field of targeted gene therapy, enabling precise modification of disease-causing genes with unprecedented efficiency. Depending on the site of genome editing, therapeutic approaches are broadly categorized into ex vivo and in vivo strategies, each with unique advantages, challenges, and clinical applications.

3.1. Ex Vivo Gene Therapy

Ex vivo gene therapy involves isolating patient-derived cells, modifying them outside the body, and subsequently reinfusing them back into the patient. This approach allows for controlled genome editing, detailed characterization of modified cells, and minimized systemic exposure, reducing off-target effects and immunogenic risks [24].

A major application of ex vivo CRISPR-based therapy is in the treatment of hemoglobinopathies, such as sickle cell disease (SCD) and β-thalassemia. In these therapies, hematopoietic stem and progenitor cells (HSPCs) are harvested from the patient and edited using CRISPR-Cas9 to disrupt the BCL11A erythroid-specific enhancer, which reactivates fetal hemoglobin (HbF) expression. This compensatory mechanism alleviates the clinical symptoms associated with defective adult hemoglobin [9,25]. Early clinical studies have demonstrated sustained HbF production and improved hematological outcomes in treated patients, highlighting the translational potential of ex vivo CRISPR therapies.

Another significant application is in cancer immunotherapy, particularly CAR-T cell therapy.

T cells extracted from patients can be engineered using CRISPR to knock out inhibitory receptors such as PD-1 or to insert chimeric antigen receptors (CARs) targeting specific tumor antigens. CRISPR-based engineering enhances the persistence, specificity, and cytotoxicity of T cells, improving therapeutic efficacy against hematologic malignancies and solid tumors [10,26].

3.2. In Vivo Gene Therapy

In vivo gene therapy entails direct delivery of CRISPR components into the patient, targeting specific tissues or organs. This approach is inherently more challenging due to immune responses against the delivery vector or Cas proteins, off-target effects in non-target tissues, and the need for high editing efficiency [27]. Common delivery platforms include viral vectors, such as adeno-associated virus (AAV) and lentivirus, which provide efficient transduction of target cells, and non-viral carriers, including lipid nanoparticles (LNPs) and polymer-based nanoparticles, which offer reduced immunogenicity and scalable production [11,28].

Notable successes of in vivo CRISPR therapies include treatment of Leber congenital amaurosis type 10 (LCA10), a hereditary retinal dystrophy, using AAV-mediated delivery of CRISPR-Cas9 to correct mutations in retinal cells, resulting in partial restoration of visual function [12,29]. Similarly, transthyretin amyloidosis (ATTR), a systemic disorder caused by mutant transthyretin protein, has been targeted using lipid nanoparticle-mediated delivery of CRISPR-Cas9 components to the liver, demonstrating durable reduction of pathogenic protein levels with minimal adverse effects [12,30].

These advancements underscore the versatility and therapeutic potential of CRISPR-Cas systems in treating a broad range of genetic disorders. While ex vivo approaches allow detailed control and reduced systemic risk, in vivo strategies enable treatment of tissues that cannot be easily extracted, expanding the scope of CRISPR-based medicine.

4. CRISPR Delivery Systems

Efficient and safe delivery of CRISPR components is a critical determinant of therapeutic success. The choice of delivery strategy affects editing efficiency, tissue specificity, immunogenicity, and off-target effects. Delivery approaches can be broadly categorized into viral and non-viral systems, each with distinct advantages and limitations [31].

4.1. Viral Delivery Systems

Viral vectors exploit the natural ability of viruses to transfer genetic material into host cells. The most commonly used viral vectors for CRISPR delivery include adeno-associated virus (AAV), lentivirus, and adenovirus.

- AAV vectors are widely employed due to their low immunogenicity, high transduction efficiency in non-dividing cells, and stable episomal expression. AAV-mediated CRISPR delivery has been successfully applied in vivo for retinal diseases, muscular dystrophies, and hepatic disorders, demonstrating therapeutic efficacy with minimal systemic toxicity [29,30,32].
- Lentiviral vectors can integrate into the host genome, enabling long-term expression of CRISPR components, which is advantageous for ex vivo gene therapy, particularly in hematopoietic stem cells. However, random integration raises concerns about insertional mutagenesis, requiring careful design and safety considerations [33].
- Adenoviral vectors can accommodate larger cargo sizes, such as base or prime editors, and can transduce a broad range of dividing and non-dividing cells. However, they elicit strong immune responses, limiting their repeated use in vivo [34].

While viral vectors achieve high transduction efficiency, immunogenicity, limited cargo capacity, and regulatory challenges remain significant barriers, motivating the development of alternative non-viral delivery strategies.

4.2. Non-Viral Delivery Systems

Non-viral delivery systems provide a safer, transient, and often more controllable method of delivering CRISPR components without genomic integration. Common strategies include lipid nanoparticles (LNPs), polymeric nanoparticles, electroporation, and direct delivery of ribonucleoprotein (RNP) complexes [28,35].

- Lipid nanoparticles (LNPs) are particularly effective for liver-targeted therapies, taking advantage of hepatic uptake after systemic administration. LNP-mediated CRISPR-Cas9 delivery has successfully reduced pathogenic protein levels in diseases such as transthyretin amyloidosis and hypercholesterolemia [30,36].
- Polymeric nanoparticles can be engineered to enhance tissue-specific targeting, stability, and endosomal escape, expanding their applicability to various organs [37].
- Electroporation and RNP delivery allow transient introduction of Cas protein and sgRNA directly into cells, reducing the duration of nuclease activity and minimizing off-target effects. This approach is widely used in ex vivo editing of T cells, HSPCs, and induced pluripotent stem cells [24,26].

4.3. Challenges and Optimization Strategies

Despite substantial progress, CRISPR delivery remains a major challenge for clinical translation. Key limitations include:

- Tissue specificity: Achieving efficient delivery to target cells while minimizing off-target exposure to other tissues.
- Immunogenicity: Pre-existing immunity to Cas proteins or viral capsids can reduce editing efficiency and induce adverse immune reactions.
- Cargo limitations: Many viral vectors have restricted capacity, limiting delivery of larger constructs such as base editors or prime editors.
- Transient vs. stable expression: Balancing therapeutic efficacy with safety concerns regarding long-term Cas9 expression.

To overcome these challenges, researchers are developing engineered viral vectors with tissue-specific promoters, next-generation LNP formulations, chemical modifications of sgRNA for stability, and self-degrading Cas proteins to improve safety and precision [28,36,38]. These strategies aim to optimize delivery efficiency while minimizing off-target effects and systemic toxicity, which is essential for successful translation of CRISPR-based therapies to clinical practice.

5. Challenges and Limitations of CRISPR-Based Gene Therapy

Despite the transformative potential of CRISPR-Cas systems, several technical, biological, and ethical challenges must be addressed before widespread clinical adoption. Understanding these limitations is critical to ensure therapeutic efficacy, patient safety, and regulatory compliance.

5.1. Off-Target Effects

One of the primary technical concerns in CRISPR-mediated gene editing is off-target activity, where the Cas nuclease induces unintended double-strand breaks at genomic sites with partial sequence complementarity to the sgRNA. These off-target mutations can lead to gene disruption, chromosomal rearrangements, or tumorigenesis, posing a significant safety risk [39,40]. Although strategies such as high-fidelity Cas9 variants, truncated sgRNAs, and in silico guide design algorithms have reduced off-target cleavage, complete elimination of unintended edits remains a challenge [41]. Base editing and prime editing technologies further mitigate this

risk by avoiding double-strand breaks, but off-target base conversions or pegRNA mispairing can still occur [22,23,42].

5.2. Immune Responses

CRISPR components, particularly Cas proteins derived from bacterial species, may trigger innate or adaptive immune responses in human patients. Pre-existing antibodies and T-cell immunity against Cas9 from Streptococcus pyogenes or Cas12a from Acidaminococcus sp. have been reported, which can reduce editing efficiency or cause inflammatory reactions [43,44]. Viral delivery vectors such as AAV also elicit immune responses, which can limit repeated dosing and complicate treatment of systemic diseases [31,32]. To overcome this, transient delivery systems like ribonucleoprotein (RNP) complexes and immune-silencing strategies are under investigation [28,36].

5.3. Delivery Efficiency and Tissue Specificity

Achieving efficient delivery of CRISPR components to target cells in vivo remains a significant challenge. Systemic administration may result in low uptake by the intended tissue, off-target transduction, or rapid clearance by the immune system [27,28]. Furthermore, tissue-specific promoters and engineered nanoparticles are required to improve targeting accuracy, particularly for organs such as the heart, brain, and lungs, where delivery barriers exist [37,38].

5.4. Ethical and Regulatory Considerations

Beyond technical challenges, CRISPR-mediated gene therapy raises ethical concerns, especially regarding germline editing, heritable modifications, and equitable access to therapies. While somatic cell editing offers therapeutic benefits without passing changes to offspring, germline applications have sparked global debate due to long-term consequences, societal implications, and potential misuse [45,46]. Regulatory frameworks are evolving to balance innovation with safety, requiring rigorous preclinical studies and careful clinical trial design.

5.5. Long-Term Effects and Safety

Limited long-term data on edited cells or tissues introduces uncertainties regarding genomic stability, unintended phenotypic consequences, and persistence of edits. Continuous monitoring and follow-up of patients in clinical trials are necessary to assess durable efficacy and safety [47]. Advanced genomic surveillance, single-cell sequencing, and sensitive off-target detection techniques are being implemented to address these concerns.

In summary, while CRISPR-Cas systems offer unprecedented opportunities for therapeutic intervention, addressing off-target effects, immune responses, delivery limitations, ethical concerns, and long-term safety is essential for safe and effective translation into clinical practice. Emerging technologies and delivery innovations are actively mitigating these challenges, moving the field closer to widespread clinical application.

6. Clinical Applications and Ongoing Trials

CRISPR-Cas systems have rapidly progressed from preclinical studies to early-stage clinical trials, demonstrating therapeutic potential in a range of genetic disorders, hematologic diseases, and cancers.

6.1. Hematologic Disorders

Ex vivo CRISPR editing of hematopoietic stem cells (HSCs) has been successfully applied to sickle cell disease (SCD) and β-thalassemia. Disruption of the BCL11A enhancer reactivates fetal hemoglobin production, alleviating disease symptoms. Recent trials report sustained HbF expression and improved hematologic outcomes in patients [25].

6.2. Ocular Disorders

In vivo CRISPR-AAV therapies are being tested for Leber congenital amaurosis (LCA10). Subretinal delivery of Cas9 and sgRNA corrects mutations in retinal cells, leading to partial restoration of visual function in early-phase trials [29].

6.3. Metabolic Disorders

Lipid nanoparticle-mediated in vivo CRISPR delivery has been employed to treat transthyretin amyloidosis (ATTR). Cas9-mediated editing in hepatocytes reduces mutant protein levels significantly, demonstrating durable therapeutic effect [30].

6.4. Cancer Immunotherapy

CRISPR-engineered CAR-T cells are being evaluated in patients with refractory hematologic malignancies. Modifications include PD-1 knockout and CAR insertion, enhancing T-cell persistence and tumor targeting [26].

6.5. Summary

Ongoing clinical trials demonstrate that both ex vivo and in vivo CRISPR strategies are feasible, safe, and show promising efficacy. However, challenges such as off-target effects, immune responses, and delivery efficiency continue to limit broader application. Continuous improvements in delivery technologies, editing precision, and patient monitoring are expected to expand the clinical utility of CRISPR-based therapies.

7. Future Perspectives

Emerging genome-editing strategies such as prime editing, base editing, and CRISPR-based epigenetic modulation significantly expand the therapeutic scope of CRISPR technologies while enhancing precision and safety. Prime editing enables highly accurate insertions, deletions, and base substitutions without inducing double-strand DNA breaks, thereby minimizing unwanted insertions or chromosomal rearrangements. Base editing allows direct conversion of specific nucleotides, offering effective treatment options for single-point mutation disorders. In parallel, CRISPR-mediated epigenetic modulation facilitates reversible regulation of gene expression without altering the underlying DNA sequence, making it particularly attractive for complex diseases and functional genomics studies.

The integration of artificial intelligence (AI)-driven sgRNA design tools has further improved targeting efficiency and reduced off-target effects by optimizing guide RNA sequences through predictive modeling. Advances in high-throughput off-target detection technologies, such as GUIDE-seq and CIRCLE-seq, enable comprehensive safety assessments prior to clinical application. Moreover, the development of next-generation delivery vectors, including engineered viral systems and biodegradable lipid nanoparticles, has enhanced tissue specificity and reduced immunogenicity. Collectively, these technological advancements are expected to accelerate the safe translation of CRISPR-based therapies from experimental settings into routine clinical practice, marking a transformative step in precision medicine.

8. Conclusion

CRISPR-Cas systems represent a transformative breakthrough in targeted gene therapy, redefining the landscape of precision medicine through their unparalleled accuracy, efficiency, and adaptability. The rapid evolution of CRISPR technologies has enabled both ex vivo and in vivo therapeutic strategies, demonstrating significant clinical success across a wide spectrum of diseases. Key conclusions and future perspectives are summarized below:

- Unprecedented Precision and Versatility: CRISPR-Cas platforms allow highly specific, programmable genome modifications, enabling precise correction of disease-causing mutations and functional regulation of target genes across diverse cell types.
- Clinical Efficacy Across Multiple Diseases: Successful applications in monogenic disorders (e.g., sickle cell disease and β-thalassemia), cancer immunotherapy (CRISPR-engineered CAR-T cells), ocular diseases, and viral infections highlight the broad therapeutic potential of CRISPR-based interventions.
- Advances in Editing Technologies: Innovations such as base editing, prime

editing, and epigenetic modulation significantly reduce unintended genomic alterations while expanding the range of treatable mutations, thereby enhancing safety and therapeutic precision.

- Progress in Delivery Platforms: Improved viral vectors, lipid nanoparticles, and tissue-specific delivery systems have increased in vivo editing efficiency while minimizing immune responses and off-target exposure.
- Remaining Challenges: Despite promising outcomes, critical challenges remain, including off-target effects, delivery efficiency, immunogenicity of Cas proteins, ethical considerations, and long-term safety monitoring.
- Role of Regulation and Ethics: Robust regulatory oversight, ethical governance, and transparent clinical evaluation are essential to ensure responsible translation of CRISPR technologies into clinical practice.
- Future of Personalized Medicine: Continued integration with AI-assisted guide RNA design, high-throughput safety screening, and patient-specific therapeutic strategies positions CRISPR-Cas systems as a cornerstone for personalized, curative treatments.

In conclusion, CRISPR-Cas-mediated gene therapy holds immense promise for transforming modern medicine. As technological refinements and clinical evidence continue to accumulate, these systems are poised to usher in a new era of safe, effective, and individualized precision therapeutics.

Acknowledgement

Authors are indebted to the University of Technology and Applied Sciences for providing the facility for conducting this research. We express our heartfelt gratitude to the Head of Department Dr. Aliya Al Alawi and Head of section Dr. Samiya Al Mabsali for the continuous encouragement in carrying out the research works in the Civil and Architectural Engineering unit. We express our gratitude to all those who cooperated with this study.

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