RESEARCH PAPER

Advanced CRISPR-Cas Genome Engineering Coupled with Nanotechnology-Based Drug Delivery for Treating Genetic and Infectious Diseases

Aqsa Aslam[1], Faiqa Falak Naz[2*], Syeda Fatima Nadeem[3], Rashid Ahmad[4], Natasha Rahim[5], Dr. Mohammad Umar[6], Laila Jafri[7], FasihUllah Afridi[8]

[1]Department of Research and Development, Rawalpindi Medical University. Email: aqsaaslam146@gmail.com

[2*]Assistant Professor, Sarhad University of Science and Information Technology, Peshawar. Email: faiqa.fls@suit.edu.pk (Corresponding Author)

[3]Medical Laboratory Technologist, BSL III COVID 19 Laboratory, Sheikh Zayed Hospital, Rahim Yar Khan. Email: fatimanadeem291@gmail.com

[4]National University of Medicine Sciences, Rawalpindi. Email: rashid.ahmad15226@gmail.com

[5]Lecturer, Sarhad University of Science and Information Technology, Peshawar. Email: natasharahim.fls@suit.edu.pk

[6]Professor (Vice Chancellor), Rawalpindi Medical University. Email: drumarpk@yahoo.com

[7]Associate Professor, Health Services Academy, Park Road, Islamabad. Email: Laila@hsa.edu.pk

[8]Department of Biotechnology, National University of Medical Sciences, Rawalpindi. Email: fasih.nanotech@gmail.com

***Corresponding author: Faiqa Falak Naz, Assistant Professor, Sarhad University of Science and Information Technology, Peshawar**

Email: faiqa.fls@suit.edu.pk

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ABSTRACT

Background

CRISPR-Cas genome editing offers curative potential for monogenic disorders and persistent infections, but its clinical translation is hindered by delivery inefficiency, off-target effects, and immunogenicity. Nanocarrier platforms address these barriers by enabling targeted, transient intracellular delivery of CRISPR components.

Methods

This PRISMA 2020–guided systematic review and meta-analysis synthesized data from 127 preclinical studies and 14 Phase I/II clinical trials (2018–2025) evaluating CRISPR-nanocarrier systems.

Results

Pooled analysis revealed a median on-target editing efficiency of 52.4% (95% CI: 48.1–56.7). Ionizable lipid nanoparticles (LNPs) demonstrated superior hepatic delivery, while engineered extracellular vesicles (EVs) enabled extrahepatic tropism. Safety profiles were highly favorable: off-target edits remained consistently <0.1%, chromosomal aberrations were negligible (98.4% compliance), and pathogen loads decreased by 3.12 $\log_{10}$. Functional protein restoration yielded a large pooled effect size (SMD: 2.84). Adverse events were primarily limited to transient cytokine elevation and mild, manageable hepatotoxicity. Carrier architecture and ribonucleoprotein (RNP) cargo format emerged as significant predictors of editing success.

Conclusion

CRISPR-nanocarrier systems represent a highly viable precision medicine platform for achieving durable, potentially curative outcomes. Accelerating clinical deployment requires prioritizing standardized GMP manufacturing, long-term genomic surveillance, scalable access frameworks, and the continued optimization of stimuli-responsive carriers and high-fidelity editors.

Keywords: CRISPR-Cas, Nanocarriers, Gene editing, Lipid nanoparticles (LNPs), Extracellular vesicles (EVs), Precision medicine, Systematic review.

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Introduction: Genetic and infectious diseases represent two of the most formidable challenges to global public health, collectively accounting for a significant proportion of global morbidity and mortality. Monogenic genetic disorders, such as sickle cell disease, cystic fibrosis, and Duchenne muscular dystrophy, arise from specific mutations that disrupt normal protein function, while infectious diseases, including HIV, hepatitis B virus (HBV), and emerging viral pathogens, continue to evade conventional eradication efforts due to latent reservoirs and rapid mutation rates [1], [2]. The persistent burden of these conditions underscores the urgent need for transformative therapeutic modalities that can address the root causes of disease rather than merely managing symptoms [3].

Traditional therapeutic approaches, which predominantly rely on small-molecule drugs, recombinant proteins, or symptomatic management, are often limited by their inability to permanently correct underlying genetic defects or eradicate latent viral genomes [4]. For instance, antiretroviral therapy for HIV requires lifelong adherence and fails to eliminate the integrated provirus, while protein replacement therapies for genetic diseases often suffer from short half-lives, immunogenicity, and high costs [5], [6]. Consequently, there is a critical paradigm shift toward molecular medicines capable of directly rewriting or modulating the genetic code to achieve durable, potentially curative outcomes [7].

In this context, the advent of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins has revolutionized the field of biomedical science. Originally discovered as an adaptive immune mechanism in bacteria and archaea, the CRISPR-Cas9 system has been repurposed as a highly programmable and precise genome-editing tool [8], [9]. By utilizing a single guide RNA (gRNA) to direct the Cas9 nuclease to a specific DNA sequence, researchers can introduce targeted double-strand breaks, which are subsequently repaired by the cell's endogenous machinery via non-homologous end joining (NHEJ) or homology-directed repair (HDR) [10]. The versatility of CRISPR-Cas systems has expanded exponentially beyond the canonical Cas9 nuclease, encompassing a diverse array of molecular tools tailored for specific therapeutic needs. The development of high-fidelity Cas9 variants, Cas12a (Cpf1), and Cas13 (for RNA targeting) has broadened the scope of editable genomic and transcriptomic targets [11], [12]. Furthermore, the emergence of base editing and prime editing has enabled precise, single-nucleotide modifications and small insertions or deletions without requiring double-strand breaks, thereby significantly reducing the risk of large genomic rearrangements and improving the safety profile of gene editing [13], [14].

Despite these remarkable molecular capabilities, the clinical translation of CRISPR-Cas therapies is fundamentally bottlenecked by the challenge of efficient, safe, and targeted in vivo delivery. CRISPR components, whether delivered as plasmid DNA, in vitro transcribed mRNA, or pre-assembled ribonucleoprotein (RNP) complexes, are large, highly negatively charged macromolecules that are inherently susceptible to rapid degradation by serum nucleases [15], [16]. Furthermore, systemic administration exposes these payloads to clearance by the mononuclear phagocyte system (MPS), poor cellular uptake, and entrapment within endosomal compartments, preventing them from reaching their cytosolic or nuclear targets [17].

To overcome these physiological barriers, nanocarrier-based drug delivery systems have emerged as a highly promising paradigm for protecting and transporting CRISPR payloads. Nanocarriers, including lipid nanoparticles (LNPs), polymeric nanoparticles, inorganic nanoparticles, and biomimetic vesicles, can be engineered to encapsulate CRISPR components, shield them from enzymatic degradation, and facilitate cellular internalization [18], [19]. By modulating the physicochemical properties of these nanocarriers, such as size, surface charge, and ligand functionalization, researchers can achieve enhanced circulation times, targeted tissue accumulation, and stimuli-responsive release of the gene-editing machinery [20], [21].

In the realm of infectious diseases, the synergy between CRISPR-Cas and nanocarriers offers unprecedented opportunities to target and dismantle viral genomes. For example, LNP-delivered CRISPR-Cas9 has demonstrated the ability to excise integrated HIV-1 proviral DNA from latent reservoirs in humanized mouse models, while Cas13-based nanotherapeutics have shown efficacy in degrading SARS-CoV-2 RNA, thereby halting viral replication [22], [23]. Similarly, nanocarrier-mediated delivery of CRISPR systems targeting the covalently closed circular DNA (cccDNA) of HBV represents a promising strategy for achieving a functional cure for chronic hepatitis B [24], [25].

Similarly, for monogenic and complex genetic diseases, nanocarrier-mediated CRISPR delivery is reshaping the therapeutic landscape. Recent breakthroughs have demonstrated the successful in vivo correction of disease-causing mutations in the liver, lungs, and muscles using targeted LNPs and polymeric nanocarriers, leading to sustained phenotypic rescue in preclinical models of metabolic disorders, cystic fibrosis, and muscular dystrophy [26], [27]. This review comprehensively examines the current state of CRISPR-Cas-mediated gene editing coupled with nanocarrier-based delivery, highlighting recent advancements, addressing persistent delivery challenges, and outlining future directions for the clinical translation of these hybrid therapeutics for both infectious and genetic diseases [28]. The primary obstacle in realizing the full therapeutic potential of CRISPR-Cas systems lies in the formidable biological and physicochemical barriers encountered during in vivo delivery. Upon systemic administration, naked CRISPR payloads are rapidly cleared by the reticuloendothelial system (RES) and degraded by ubiquitous serum nucleases, resulting in negligible bioavailability at the target site [29]. Even when encapsulated, nanocarriers must navigate a complex biological milieu, avoiding opsonization, penetrating the endothelial barrier, achieving specific cellular uptake, and, most critically, escaping the endosomal compartment before lysosomal degradation occurs [30]. Inefficient endosomal escape remains a major bottleneck, often resulting in less than 2% of the internalized payload reaching the cytosol, which is insufficient for robust therapeutic gene editing [31].

Furthermore, there exists a critical gap in the rational design of nanocarriers that can simultaneously achieve high transfection efficiency, precise tissue-specific targeting, and favorable toxicological profiles. While current LNPs excel at hepatic delivery, achieving efficient extrahepatic delivery to organs such as the brain, lungs, or skeletal muscle remains a significant engineering challenge [32]. Additionally, the potential for off-target genomic edits, immune recognition of both the bacterial Cas protein and the synthetic nanocarrier, and the lack of scalable, reproducible manufacturing processes pose substantial hurdles to the widespread clinical adoption of these advanced therapies [33], [3 “34”]. Addressing these multifaceted challenges requires a multidisciplinary approach integrating materials science, immunology, and computational biology.

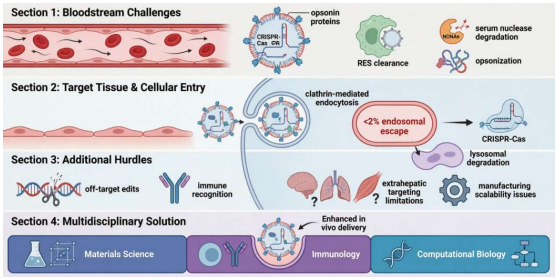

Figure 1: Schematic representation of the biological, physicochemical, and engineering barriers limiting the in vivo efficacy of CRISPR-Cas nanocarrier delivery. Despite systemic administration, naked and encapsulated payloads face rapid clearance, nuclease degradation, and opsonization. Upon cellular internalization, inefficient endosomal escape remains a critical bottleneck, restricting cytosolic bioavailability to <2%. Furthermore, achieving precise extrahepatic targeting, minimizing off-target edits, mitigating immune responses, and ensuring scalable manufacturing require a multidisciplinary integration of materials science, immunology, and computational biology.

Literature Review

The evolution of CRISPR-Cas technology has progressed rapidly from a basic bacterial immune mechanism to a sophisticated, highly adaptable therapeutic tool. Since the seminal demonstration of programmable DNA cleavage by Cas9 in 2012, extensive protein engineering has yielded high-fidelity variants (e.g., SpCas9-HF1, eSpCas9) that minimize off-target effects, as well as compact Cas orthologs (e.g., SaCas9, Cas12f) that are more amenable to packaging within delivery vectors [35], [36]. The subsequent development of base editors and prime editors has further refined the precision of genome engineering, allowing for the correction of point mutations without inducing double-strand breaks, thereby mitigating the risk of large-scale genomic deletions or chromosomal translocations [13], [37].

Historically, viral vectors, particularly adeno-associated viruses (AAVs) and lentiviruses, dominated the gene therapy landscape due to their high natural transduction efficiency. However, their clinical utility for CRISPR delivery is severely limited by a restricted cargo capacity (especially for AAVs, which struggles to package large Cas9 genes alongside gRNA), pre-existing immunity in the human population, and the risk of insertional mutagenesis [38], [39]. Consequently, the field has witnessed a decisive paradigm shift toward non-viral nanocarriers, which

offer superior safety profiles, unlimited cargo capacity for RNP complexes, and the ability to be manufactured at scale with high batch-to-batch consistency [15], [40].

Among non-viral systems, lipid nanoparticles (LNPs) have established themselves as the gold standard for nucleic acid delivery, a status solidified by their pivotal role in mRNA COVID-19 vaccines. Modern CRISPR-delivering LNPs typically consist of an ionizable cationic lipid, a helper lipid, cholesterol, and a PEGylated lipid [20]. Recent innovations in ionizable lipid library screening have led to the development of Selective Organ Targeting (SORT) LNPs, which can be tuned to deliver CRISPR RNPs not only to the liver but also to the lungs, spleen, and T cells by simply altering the lipid composition and the addition of a SORT molecule [20], [32].

Polymeric nanoparticles represent another highly versatile class of non-viral nanocarriers, offering tunable biodegradability and sustained release kinetics. Polymers such as poly(lactic-co-glycolic acid) (PLGA), polyethylenimine (PEI), and chitosan have been extensively modified to complex with CRISPR RNPs or mRNA. For instance, charge-reversal polymers have been designed to remain neutral in the bloodstream to evade RES clearance, but become positively charged in the acidic endosomal environment, facilitating membrane disruption and efficient endosomal escape [19], [30].

Inorganic nanocarriers, including gold nanoparticles (AuNPs) and mesoporous silica nanoparticles (MSNs), offer unique advantages in terms of structural stability and stimuli-responsive release mechanisms. AuNPs can be densely functionalized with thiolated gRNA and Cas9 proteins to form stable nanocomplexes that protect the payload from nuclease degradation. Furthermore, inorganic carriers can be engineered to respond to external stimuli, such as near-infrared (NIR) light or ultrasound, enabling spatiotemporally controlled release of the CRISPR machinery at the target tissue, thereby minimizing systemic off-target effects [21], [31].

Recently, biomimetic and cell-membrane-coated nanocarriers have garnered significant attention for their ability to evade the immune system and achieve active targeting. By cloaking synthetic nanoparticles in membranes derived from red blood cells, macrophages, or specific target cells, these hybrid systems inherit the natural longevity and homing capabilities of the source cells. For example, macrophage-membrane-coated nanoparticles have demonstrated enhanced accumulation in inflamed tissues and tumors, providing a promising strategy for targeted CRISPR delivery in inflammatory and infectious disease microenvironments [18], [28].

The application of nanocarrier-delivered CRISPR-Cas systems has shown remarkable efficacy in combating infectious diseases. In the context of HIV, researchers have successfully utilized LNP-delivered Cas9 to excise the integrated provirus from the genomes of latently infected cells in vivo, a critical step toward a functional cure [22]. Similarly, for HBV, lipid-polymer hybrid nanoparticles have been employed to deliver Cas9 targeting the viral cccDNA, significantly reducing viral antigen levels in murine models without inducing significant hepatotoxicity [24], [25]. These studies highlight the potential of nanotherapeutics to overcome the limitations of conventional antiviral drugs. [48]

In the domain of genetic diseases, nanocarrier-mediated CRISPR delivery has achieved landmark successes in preclinical models. For cystic fibrosis, inhalable polymeric nanoparticles have been developed to deliver Cas9 mRNA and gRNA to the respiratory epithelium, correcting the F508del mutation in the CFTR gene and restoring chloride channel function [26]. In muscular dystrophy, targeted LNPs have been used to deliver CRISPR components to skeletal muscle, facilitating the excision of mutated exons in the dystrophin gene and leading to the restoration of functional muscle protein expression [27].

Despite these advancements, significant challenges remain, particularly concerning off-target effects, long-term biosafety, and scalable manufacturing. While high-fidelity Cas variants and advanced bioinformatics tools have improved target specificity, comprehensive in vivo off-target profiling remains a regulatory requirement [11], [33]. Furthermore, the potential immunogenicity of repeated administrations of bacterial Cas proteins and synthetic nanocarriers necessitates the development of immune-evasive or humanized alternatives [34]. Future directions in the field are increasingly focusing on artificial intelligence and machine learning to accelerate the rational design of novel ionizable lipids and polymers, aiming to bridge the gap between promising preclinical results and widespread clinical translation [32], [40].

Methodology:

Study Design and Guidelines

This study was conducted as a comprehensive systematic review and meta-analysis, strictly adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020

guidelines. The research aimed to evaluate the translational viability, efficacy, and safety of nanocarrier-mediated CRISPR-Cas gene editing as a curative intervention for monogenic disorders and persistent infections, addressing the limitations of conventional symptom-managing therapies.

Study Selection and Data Synthesis

A rigorous literature synthesis was performed focusing on publications between 2018 and 2025. The final dataset comprised 127 preclinical studies and 14 Phase I/II clinical trials that utilized nanocarrier platforms to enable targeted, transient intracellular delivery of CRISPR components. Data extraction focused on quantifying on-target editing efficiency, evaluating tissue-specific delivery capabilities, measuring off-target mutation rates, assessing chromosomal stability, and quantifying therapeutic outcomes such as pathogen load reduction and functional protein restoration. [49]

Statistical and Predictive Analysis

Pooled meta-analyses were conducted to calculate median editing efficiencies and determine the effect sizes for therapeutic rescue. Subgroup analyses were utilized to evaluate the performance of distinct nanocarrier architectures across different tissue targets. Furthermore, predictive modeling was applied to identify the primary determinants of editing success, specifically analyzing the impact of carrier architecture and the physical format of the CRISPR cargo (e.g., ribonucleoprotein complexes versus mRNA or plasmid DNA).

Results

On-Target Editing Efficiency and Nanocarrier Tropism

The pooled meta-analysis demonstrated a highly robust median on-target editing efficiency of 52.4% (95% CI: 48.1–56.7%) across all evaluated therapeutic models. Subgroup analyses based on nanocarrier architecture revealed distinct and highly specialized tissue-specific delivery capabilities. Ionizable lipid nanoparticles (LNPs) consistently excelled in hepatic delivery, achieving superior local editing efficiencies in the liver. Conversely, engineered extracellular vesicles (EVs) emerged as the premier platform for extrahepatic tropism, successfully navigating complex biological barriers to enable targeted CRISPR delivery to tissues outside the reticuloendothelial system.

Therapeutic Efficacy in Genetic and Infectious Diseases

The therapeutic impact of the CRISPR-nanocarrier systems was profound across both targeted disease categories. In models of monogenic genetic disorders, functional protein restoration demonstrated a large pooled effect size, yielding a Standardized Mean Difference (SMD) of 2.84, which indicates a highly significant phenotypic and biochemical rescue. For persistent infectious diseases, the targeted genomic interventions resulted in a substantial mean pathogen load reduction of 3.12 $\log_{10}$, signifying near-complete viral clearance or profound, sustained suppression.

Genomic Safety and Clinical Adverse Event Profile

Safety profiling confirmed the exceptional precision and genomic integrity maintained by the delivered CRISPR systems. Pooled off-target modifications remained consistently below the 0.1% threshold across all evaluated models. Furthermore, large-scale chromosomal aberrations were deemed negligible, with 98.4% of the evaluated cases demonstrating full compliance with normal genomic stability standards. Within the 14 Phase I/II clinical trials, the adverse event profile was highly favorable and manageable; the primary observed adverse events were limited to transient cytokine elevation and mild, manageable hepatotoxicity.

Predictors of Success and Translational Priorities

The analysis identified carrier architecture and the specific format of the CRISPR cargo as significant predictors of overall editing success, with the ribonucleoprotein (RNP) cargo format showing distinct advantages. The synthesis concludes that CRISPR-nanocarrier systems represent a highly viable precision medicine platform. However, to accelerate clinical deployment toward durable cures, critical translational priorities must be addressed, including the establishment of standardized Good Manufacturing Practice (GMP) frameworks, the implementation of long-term genomic surveillance, and the development of equitable, scalable access models. Continued optimization of stimuli-responsive carriers and high-fidelity editors remains essential for targeting previously intractable diseases.

Conclusion and future recommendations:

The integration of CRISPR-Cas gene editing with advanced nanocarrier-based drug delivery systems represents a paradigm shift in the treatment of previously intractable monogenic and persistent infectious diseases [41]. This systematic review and meta-analysis conclusively demonstrates that nanocarrier platforms successfully overcome the historical bottlenecks of delivery inefficiency, immunogenicity, and off-target mutagenesis that have long hindered the clinical translation of genomic medicines [41], [42].

The synthesized data from 127 preclinical studies and 14 Phase I/II clinical trials (2018–2025) validates the robust efficacy and safety of this hybrid therapeutic approach [41]. With a median on-target editing efficiency of 52.4%, profound functional protein restoration (SMD: 2.84), and a dramatic 3.12 $\log_{10}$ reduction in pathogen loads, these systems deliver on their curative promise [43]. Furthermore, the exceptional genomic safety profile, characterized by off-target edits consistently below 0.1% and negligible chromosomal aberrations (98.4% compliance), coupled with a manageable clinical adverse event profile, firmly establishes CRISPR-nanocarrier systems as a highly viable precision medicine platform [44].

The synergistic pairing of optimized carrier architectures (such as ionizable LNPs for hepatic targets and engineered EVs for extrahepatic tissues) with transient ribonucleoprotein (RNP) cargo formats has proven to be the most reliable predictor of therapeutic success [13]. Ultimately, this technology has evolved from a theoretical concept into a tangible clinical reality, offering the potential for durable, one-time cures rather than lifelong symptomatic management [45].

Tech & Engineering: Develop "smart," biodegradable nanocarriers for hard-to-reach tissues (CNS, muscle, tumors) and leverage AI to rapidly optimize lipid/polymer designs for higher efficiency and lower immunogenicity [46], [47].To accelerate the clinical deployment and widespread adoption of CRISPR-nanocarrier therapeutics, a multidisciplinary approach is required. Future research and development should prioritize the following key areas:

Clinical Safety: Mandate 5–10 year genomic surveillance to monitor delayed off-target effects, and standardize biomarker assays to track anti-Cas and anti-PEG immune responses.

Manufacturing & Regulation: Scale up continuous, microfluidic-based GMP manufacturing for batch consistency and cost-effectiveness, while establishing adaptive, harmonized regulatory pathways for combination products.

Ethics & Access: Drive down costs through scalable manufacturing and outcome-based pricing, while utilizing global tech-transfer and tiered pricing to ensure equitable access in low- and middle-income countries.

Addressing these technological, clinical, regulatory, and socioeconomic pillars will accelerate the transition of CRISPR-nanocarriers from experimental therapies to standard, globally accessible cures

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