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Revolutionizing antiviral therapies: the promise of nucleic acid-based interventions

Senthil Thirusangu[1], Janet Prameela Dsouza[2], Sumana Mukhopadhyay[3], Abdullahi Saminu[4], Tathagata Jha[5], Prabhat[6], Aroop Mohanty[7*]

[1] Teerthakar Mahaveer University, Moradabad, India.
[2] Department of Medical Surgical Nursing, Manipal College of Nursing, Manipal Academy of Higher Education, Manipal, India.
[3]Department of Community Medicine, Grant Government Medical College and JJ Hospital, Mumbai, India.
[4] Department of Environmental Health Sciences, Faculty of Allied Health Sciences, Bayero University Kano, Nigeria.
[5] Medical College and Hospital, West Bengal University of Health Sciences, Kolkata, India.
[6] Department of Biochemistry, All India Institute of Medical Sciences, Gorakhpur, India.
[7] Department of Microbiology, All India Institute of Medical Sciences, Gorakhpur, India.

*Correspondence: aroopmohanty7785@yahoo.com

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Evidence in Context

- Examines nucleic acid-based interventions: ASOs, siRNAs, CRISPR, mRNA vaccines.
- Highlights advancements in delivery systems like lipid nanoparticles.
- Discusses the clinical potential and challenges, including safety concerns and genotoxicity.
- Notes rapid development and effectiveness of mRNA vaccines during the COVID-19 pandemic.
- Calls for continued innovation in efficacy, safety, and application of these therapies

To view Article

Abstract

Nucleic acid therapeutics are emerging as transformative agents in antiviral treatment, leveraging precise genetic interventions to combat viral infections. This mini-review examines key modalities including Antisense Oligonucleotides (ASOs), Small Interfering RNAs (siRNAs), CRISPR-based gene editing, and mRNA vaccines. These approaches utilize cellular mechanisms to inhibit viral replication and offer targeted gene silencing, crucial for addressing rapidly mutating viruses. The review highlights advancements in delivery systems, particularly lipid nanoparticles, and discusses the clinical potential and challenges of these therapies, such as safety concerns related to immune responses and genotoxicity. It also underscores the rapid development and effectiveness of mRNA vaccines demonstrated during the COVID-19 pandemic, reflecting the adaptability and potential of nucleic acid therapies. The review calls for continued innovation and multidisciplinary research to enhance the efficacy, safety, and clinical application of these promising antiviral strategies.

Keywords: *Nucleic acid therapeutics, CRISPR, antisense oligonucleotides, lipid nanoparticles, Small Interfering RNA*

Introduction

Nucleic acid therapeutics represent a cutting-edge domain within the broader context of antiviral treatments, leveraging the intrinsic mechanisms of genetic manipulation to directly combat viral pathogens [1,2]. These innovative therapies, including antisense oligonucleotides [3], small interfering RNAs [4], CRISPR-based gene editing [5], and mRNA vaccines [6], have garnered significant attention due to their potential to provide precise, targeted interventions against viral genomes. By intervening at the molecular level, nucleic acid therapies offer a novel approach to antiviral treatment, distinct from traditional methods that often focus on inhibiting viral replication enzymes and structural proteins [7,8].

Despite the groundbreaking advancements in nucleic acid therapeutics, numerous viral diseases continue to pose significant treatment challenges, primarily due to the rapid development of resistance to existing therapies [9]. This situation underscores a critical gap in the antiviral treatment landscape, where conventional drugs fall short, thereby necessitating the exploration and development of alternative therapeutic strategies. Nucleic acid therapeutics emerge as a promising solution, aiming not only to manage viral infections but also to potentially achieve curative

Outcomes by targeting the genetic material of viruses directly [10]. However, realizing the full potential of these therapies requires overcoming significant hurdles related to delivery systems, specificity, immune evasion, and the cost of therapy.

This review seeks to delve into the recent developments and current state of nucleic acid therapeutics in the context of viral infections. It aims to synthesize insights from various research studies and clinical trials that have highlighted both the successes and the ongoing challenges faced by these therapeutic strategies [3, 11]. Furthermore, the review will critically analyze the mechanisms of action of these therapies, their efficacy and safety profiles, and the innovative strategies being developed to enhance their delivery and precision. By providing a comprehensive overview of nucleic acid therapeutics, this review will contribute to the broader understanding of their potential impact on the field of antiviral treatment and will outline the necessary steps forward to address the existing research gaps and clinical challenges.

Types of nucleic acid therapeutics

Antisense oligonucleotides

ASOs function by exploiting the natural cellular mechanisms of gene expression regulation, providing a precise method to modulate genetic outputs. These synthetic, single-stranded DNA or RNA molecules are designed to be complementary to specific mRNA sequences of interest [12]. Upon administration, ASOs hybridize to their target mRNA through Watson-Crick base pairing, forming DNA-RNA or RNA-RNA duplexes (Figure 1A) [13]. This binding can lead to one of several outcomes depending on the ASO design and target sequence. One primary mechanism involves the recruitment of RNase H, an endogenous enzyme. When an ASO binds to mRNA, it forms a hybrid duplex that RNase H recognizes and cleaves the mRNA component of the duplex. This enzymatic cleavage leads to mRNA degradation, effectively reducing the translation of the target protein [14]. Another mechanism by which ASOs exert their effects is by steric blocking. Here, the ASO binds to mRNA but does not lead to its degradation [15]. Instead, it physically obstructs the translation machinery or the processing of pre-mRNA, thereby preventing the synthesis of the protein. This method is useful for modulating processes such as splicing, where the ASO can alter splice site selection to promote or inhibit the inclusion of specific exons in the mature mRNA [16]. Additionally, ASOs can be modified to enhance their stability, cellular uptake, and binding affinity. Modifications such as Locked Nucleic Acids (LNAs) and phosphorothioate backbones are common, helping to protect ASOs from nucleases and improve their pharmacokinetic properties [17]. This allows for more effective and sustained gene silencing with potential applications across a range of diseases, from viral infections to genetic disorders. Through these diverse mechanisms, ASOs provide a versatile platform for therapeutic gene regulation, allowing for the targeted silencing of detrimental genes with high specificity and minimal off-target effects [18]. This targeted approach is especially significant in the field of antiviral therapies, where precision and adaptability to rapidly mutating viruses are crucial. Research by Dauksaite et al. showcases the utilization of locked nucleic acid gapmers, a type of ASO, to target the RNA genome of SARS-CoV-2, achieving a significant reduction in viral load in vitro by up to 96% [19]. Similarly, Lu et al. developed ACE2-specific ASOs to reduce SARS-CoV-2 infection rates by downregulating ACE2 expression, a critical receptor for the virus, demonstrating potent in vitro and in vivo efficacy [3]. The clinical trial outcomes, though preliminary, have shown promising results, necessitating further studies to establish long-term efficacy and safety profiles [20, 21]. Collectively, these advances underscore the transformative potential of ASOs in providing a new arsenal against viral pathogens, paving the way for broader applications in infectious disease management.

Small Interfering RNA

SiRNA operates through a sophisticated cellular mechanism known as RNA Interference (RNAi), which plays a crucial role in gene regulation and antiviral defense. This process begins when double-stranded RNA molecules are cleaved into short 21-23 nucleotide siRNA duplexes by the enzyme Dicer (Figure 1B) [4]. Each siRNA duplex consists of a sense strand and an antisense strand, the latter of which is essential for the mechanism's specificity. The antisense strand of the siRNA is then incorporated into the RNA-induced Silencing Complex (RISC) [22]. The RISC is an assembly of proteins that facilitates the interaction between siRNA and its target mRNA. The siRNA serves as a guide for RISC, directing it to a complementary sequence on the mRNA transcript. Upon binding, the Argonaute protein within RISC utilizes its endonuclease activity to cleave the mRNA,

Thereby preventing its translation into protein. This cleavage is precise, occurring between specific nucleotides relative to the siRNA binding site, leading to effective and targeted gene silencing [23].

Notable successes of siRNA-based interventions include experimental therapies against respiratory viruses like SARS-CoV-2. For instance, Gonzalez et al. demonstrated that siRNAs could be designed to target COVID-19 variants effectively, employing bioinformatics tools to identify sequences capable of binding to viral mRNA and halt its function [24]. This approach has shown promise in vitro, with siRNAs significantly reducing viral load, although challenges in delivery and stability persist. Another critical area where siRNA has shown potential is in treating Hepatitis B and C viruses [25]. Clinical trials have explored siRNAs that target viral replication mechanisms, offering a new avenue for therapy where traditional treatments might fail or lead to resistance [26]. The precision of siRNA in silencing specific viral genes without affecting other cellular processes underscores its potential as a powerful antiviral agent [27].

CRISPR and gene editing

CRISPR-Cas9, an adaptive immune system in bacteria, has been co-opted into a powerful gene-editing tool that operates with remarkable precision and efficiency [28]. This system is predicated on the CRISPR-associated protein 9 (Cas9), an endonuclease that introduces double-stranded breaks at specific locations within the DNA, guided by a custom-designed RNA sequence known as the Guide RNA (gRNA) (Figure 1C) [2]. The gRNA is engineered to be complementary to the target DNA sequence, ensuring specificity. Upon introduction into the cell, the Cas9-gRNA complex binds to the DNA through base pairing between the gRNA and the target DNA sequence. The presence of a Protospacer Adjacent Motif (PAM) sequence immediately downstream of the target site is crucial for Cas9 recognition and subsequent DNA cleavage [29]. Once bound, Cas9 induces a Double-stranded Break (DSB) in the DNA at the target site. The cell's natural DNA repair mechanisms then engage to mend the break. There are two primary pathways through which this repair can occur: Non-homologous End Joining (NHEJ) and Homology-directed Repair (HDR) [30]. NHEJ, the more error-prone of the two, often leads to insertions or deletions (indels) at the repair site, which can disrupt gene function—useful for gene knockout studies [31]. Conversely, HDR, which is more precise, allows for the introduction of specific mutations or gene inserts by providing a DNA repair template with the desired sequence changes [32]. This precision and versatility make CRISPR-Cas9 a powerful tool for gene editing across various biological systems and applications.

Current research has demonstrated CRISPR's potential in combating an array of viral diseases. For instance, Dash et al. utilized CRISPR to edit the CCR5 receptor and HIV-1 proviral DNA, which significantly reduced the viral load in humanized mice models [33]. This approach not only suppresses the virus but potentially offers a durable cure by excising the integrated viral genome. Similarly, studies like those by Badu et al. highlight CRISPR's role in enhancing innate immune responses against viruses like Zika, suggesting broader applications in modifying host-virus interactions and improving antiviral immunity [34]. In future, the potential applications of CRISPR in virology are vast. Beyond targeting specific viruses, CRISPR could be used to engineer viral resistance in populations or develop more effective viral vaccines.

Messenger RNA vaccines

Messenger RNA (mRNA) vaccines represent a transformative leap in vaccine development, particularly highlighted by their critical role during the COVID-19 pandemic [35, 36]. These vaccines utilize mRNA to instruct cells to produce a protein that is identical or similar to a protein found on the surface of a pathogen, initiating an immune response without exposing the recipient to the actual pathogen (Figure 1D) [6]. This technology capitalizes on the cell's natural processes: the mRNA, once inside the body’s cells, is used as a template by the ribosomes to synthesize the pathogen's antigen. The immune system then recognizes this antigen as foreign, mounts an immune response, and develops a memory of the pathogen, preparing the body to fight the real thing in future encounters [37]. The deployment of mRNA vaccines against COVID-19 has provided clear examples of the rapid scalability and efficacy of this technology. Vaccines like Pfizer-BioNTech and Moderna were developed and distributed at unprecedented speeds, demonstrating significant efficacy in preventing severe disease, as corroborated by extensive clinical trials and real-world studies [38]. This success has not only curbed the spread of the virus but also showcased the potential of mRNA vaccines to be rapidly reprogrammed to tackle new variants of the virus, a flexibility not as readily available in traditional vaccine platforms [39].

Moreover, the impact of mRNA vaccines extends beyond infectious diseases. Research is exploring their application in treating and preventing complex conditions like cancer, where vaccines can be tailored to produce antigens specific to an individual's tumor, personalizing treatment in a way that was not previously possible [40]. This burgeoning field, exemplified by ongoing trials and studies, continues to explore the full potential of mRNA technologies, hinting at a new era where vaccines and therapeutics are quickly adaptable, highly specific, and broadly effective against a range of diseases.

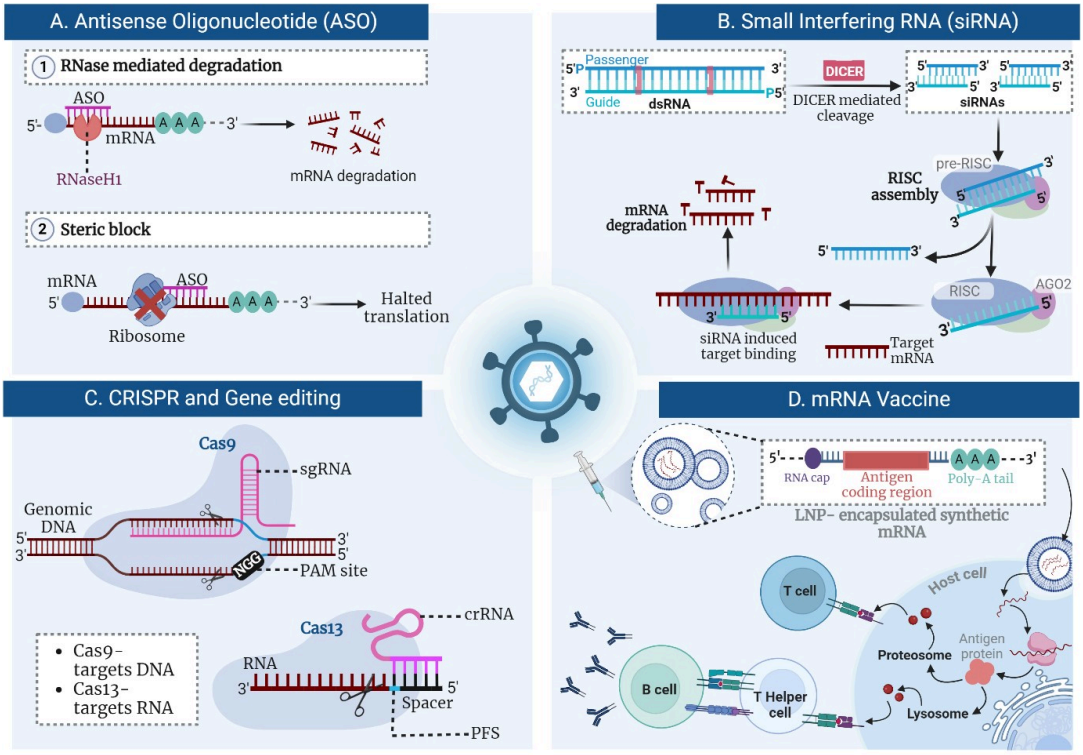

Figure 1:Mechanisms of nucleic acid-based therapeutic interventions: ASOs, siRNA, CRISPR, and mRNA vaccines

Advancements in delivery systems

Lipid nanoparticles

Lipid Nanoparticles (LNPs) have emerged as a pivotal advancement in the delivery of nucleic acid therapies, addressing critical challenges associated with the stability, delivery efficiency, and cellular uptake of these therapeutic agents [40]. The core mechanism by which LNPs enhance the delivery of nucleic acids like mRNA, siRNA, and CRISPR/Cas components involves encapsulating the nucleic acids within a lipid bilayer, protecting them from enzymatic degradation in the bloodstream and enhancing their absorption into cells [41-43].

Recent advancements in LNP technology have focused on optimizing the lipid composition to improve targeting specificity and reduce immunogenic responses (Figure 2). For instance, studies such as those by Im et al. have demonstrated the use of ionizable lipid nanoparticles for the efficient delivery of CRISPR/Cas9 ribonucleoproteins, highlighting their potential in achieving high gene editing efficiencies in vivo, particularly in cancer therapy [44]. This study exemplifies how tweaking the ionizable lipid content can significantly enhance the endosomal escape of the delivered genetic material, thereby increasing the efficacy of gene editing within target cells. Moreover, the development of LNPs that can bypass certain physiological barriers presents another significant leap forward. For instance, Kasiewicz et al. successfully employed GalNAc-modified LNPs for targeted delivery to the liver via receptors that are independent of the traditional low-density lipoprotein pathways [45]. This approach is particularly advantageous for individuals with genetic variations affecting conventional pathways, showcasing the LNPs' versatility and adaptability to diverse clinical needs. These advancements underline the transformative potential of LNPs in the realm of gene therapy, offering more effective and safer delivery mechanisms for nucleic acid-based therapies and opening new avenues for treating a wide range of diseases, from genetic

Disorders to cancers and infectious diseases.

Viral vectors

Viral vectors have become a cornerstone in the delivery of nucleic acid-based therapies due to their high efficiency in gene transfer and persistent expression in host cells [46]. These vectors are engineered from viruses, such as adenovirus, lentivirus, and Adeno-associated Virus (AAV), which have been modified to carry therapeutic genes while having their pathogenic elements removed to ensure safety (Figure 2.) [47]. Their use in clinical settings has expanded the possibilities for treating genetic disorders, cancers, and infectious diseases by enabling precise gene editing or gene replacement strategies. One notable application of viral vectors is in the delivery of the CRISPR/Cas9 system for cancer therapy [48]. For instance, the integration of lentiviral vectors to deliver CRISPR components has shown promise in targeting and editing genes implicated in cancer progression, offering a potential for durable and effective treatments [46]. Similarly, the CLEAR strategy, utilizing viral vectors for delivering CRISPR/Cas9 to inhibit the proliferation of Herpes Simplex Virus in infected cells, exemplifies the therapeutic potential of these vectors in antiviral strategies [49].

However, despite their effectiveness, the clinical use of viral vectors is not devoid of challenges. Issues such as immunogenicity, potential for insertional mutagenesis, and difficulty in manufacturing at scale can limit their utility [50]. These challenges necessitate ongoing research and development to optimize viral vector systems for safer and more effective delivery in diverse therapeutic contexts. The evolution of these vectors continues to be instrumental in pushing the boundaries of gene therapy, offering hope for more robust and accessible treatments for complex diseases.

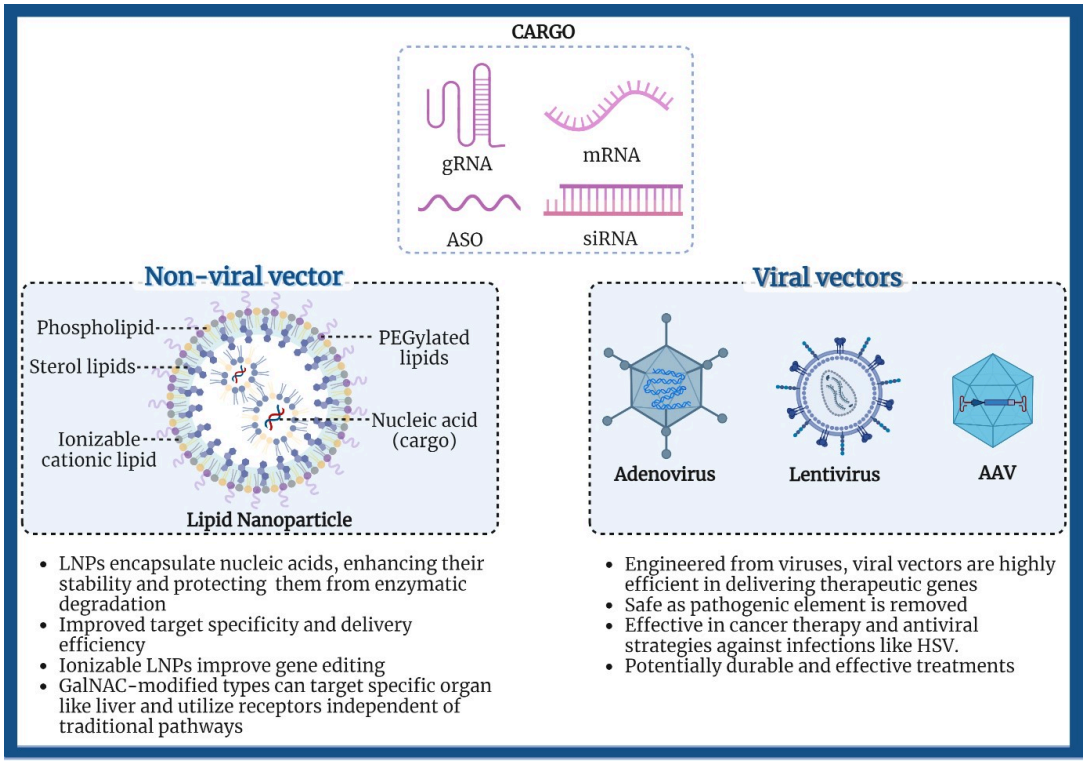

Figure 2: Comparative overview of non-viral and viral vectors in gene delivery systems

Challenges and limitations

In the expanding field of nucleic acid therapeutics for viral infections, several pivotal challenges and limitations hinder their widespread clinical application. Foremost among these is the concern for safety. Gene editing tools and the immunogenic potential of delivery vectors raise significant safety questions, especially given the incidental introduction of non-therapeutic nucleic acids within Recombinant AAV (rAAV) vectors, which can enhance the immunogenic risk associated with these

Therapies [47]. This complexity is exacerbated when considering the diverse immunological landscapes of different patient populations, necessitating a careful evaluation of immunogenic responses.

Moreover, the delivery of these therapeutic agents presents substantial obstacles. Effective and targeted delivery of nucleic acids, particularly to specific tissues, remains a critical challenge [51]. This issue is exemplified in the context of HIV/AIDS treatment, where RNA-based therapies need precise delivery to T-lymphocyte-rich tissues to effectively target latent infections [27]. The inherent instability and negative charge of RNA molecules complicate their delivery, requiring the use of sophisticated systems such as lipid nanoparticles [41]. However, these systems themselves must be meticulously designed to optimize targeting specificity and transfection efficiency while minimizing potential immune reactions [6]. Lastly, the cost and accessibility of nucleic acid therapeutics pose significant barriers to their adoption. The high costs associated with the development, production, and distribution of these therapies restrict their availability, particularly in low-resource settings. Furthermore, some therapies require extensive cold-chain logistics for distribution, which can be prohibitive in regions lacking the necessary infrastructure [52].

Discussion

The field of nucleic acid therapeutics for viral infections has seen significant advances, marked by notable success stories and a promising trajectory for future developments. Recent breakthroughs, particularly in CRISPR-based gene editing, highlight the potential for these technologies to offer lasting solutions to longstanding challenges in viral disease management. For instance, dual CRISPR-Cas9 gene editing has demonstrated the potential to not only suppress but potentially eliminate HIV-1 from humanized mouse models by precisely excising integrated proviral DNA [33]. This suggests a pathway toward not just treating but curing HIV/AIDS, showcasing the profound impact of gene editing technologies. Additionally, ASOs targeting the SARS-CoV-2 RNA genome have shown up to 96% reduction in viral load in vitro, which reinforces the role of ASOs in managing not only chronic viral infections but also in addressing acute viral outbreaks [19]. The effectiveness and practicality of nucleic acid therapies such as ASOs and siRNAs vary depending on the viral infection being targeted [27]. However, their delivery remains complex and requires sophisticated systems such as lipid nanoparticles for efficient cellular uptake [53]. On the other hand, CRISPR-based therapies provide more durable responses and have the potential for broader application in curing viral diseases by removing the virus from the host's genome [48]. Yet, these therapies also face significant challenges, including ensuring precise delivery, avoiding unintended genetic modifications, and mitigating potential immune responses [54].

The future direction of nucleic acid therapeutics in viral infections is rich with opportunities. Enhancing delivery mechanisms remains a critical focus, with lipid nanoparticles showing promise in improving the cellular uptake and overall distribution of these therapeutic molecules. There is also an ongoing need to refine the specificity and safety of CRISPR-based interventions to prevent off-target effects that could lead to unintended consequences [29]. Moreover, expanding the scope of these therapies to address a wider range of viruses and exploring their potential for prophylactic use could revolutionize how viral infections are managed globally. Integrating advanced technologies such as machine learning and bioinformatics could further enhance the design and optimization of nucleic acid therapeutics, allowing for treatments that are tailored to individual genetic profiles and specific characteristics of viral strains [55]. Such precision medicine approaches could lead to more effective and personalized therapeutic solutions, significantly impacting patient outcomes in viral disease treatment.

Conclusion

This review underscores the transformative potential of nucleic acid therapeutics in antiviral treatment, emphasizing recent advancements and ongoing challenges. The precision of ASOs, siRNAs, CRISPR interventions, and mRNA vaccines marks a significant departure from traditional therapies, offering new strategies against viral infections. Future research should focus on overcoming delivery challenges, enhancing specificity, and broadening the application to various viruses. Continued innovation in delivery technologies and the integration of computational tools are essential for optimizing these therapies and expanding their clinical utility.

Abbreviations

ASOs: Antisense oligonucleotides

Cas9: CRISPR-associated protein 9

LNPs: Lipid nanoparticles

LNAs: Locked nucleic acids

NHEJ: Non-homologous end joining

SiRNAs: Small interfering RNAs

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