World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(REVIEW ARTICLE)

Check for updates

Gene editing approaches to combat infectious diseases: Therapeutic innovations and diagnostic platforms

Fagbemi Oluwaseyi Ajibola [1,*], Toluwani Samuel Ogunmoyero [2], Mustapha Lawal [3], Iyiola Aanuoluwa Temitayo [4], Olonma Alexandra Nzei [5], Taiwo Emmanuel Olowodasa [6] and Precious Airebanmen Otoibhili [7]

[1] *Department of Human Anatomy, College of Medicine, Federal University Lokoja, Kogi State, Nigeria.*
[2] *Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomosho, Oyo, Nigeria.*
[3] *Department of Microbiology, Kebbi State University of Science and Technology Aleiro, Nigeria.*
[4] *Department of Biochemistry, African Centre of excellence of Mycotoxins and Food safety Research, Federal university of Technology, Minna, Nigeria.*
[5] *Department of Microbiology, University of Nigeria, Nsukka.*
[6] *Department of Basic Science and Research, Intercounty Centre for Oral Health for Africa, Jos, Nigeria.*
[7] *Department of Anatomy, Ambrose Alli University, Ekpoma, Edo, Nigeria.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 22(02), 389–418

Publication history: Received on 04 April 2025; revised on 20 May 2025; accepted on 22 May 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.22.2.0505

Abstract

Infectious diseases remain a formidable global health burden, exacerbated by rising antimicrobial resistance, emerging viral outbreaks, and the persistent limitations of conventional therapeutic and diagnostic strategies. This review explores the revolutionary potential of gene editing technologies—most notably CRISPR-Cas systems, base editing, and prime editing—as transformative tools in the fight against infectious diseases. By elucidating the molecular mechanisms and precision capacities of these platforms, the article delineates how gene editing enables not only the direct eradication of viral and bacterial pathogens but also the modulation of host genetic responses to enhance immunity. Key applications include CRISPR-based excision of latent HIV reservoirs, base editing for hereditary viral susceptibility correction, and prime editing for monogenic disease interventions, all backed by compelling preclinical and early clinical evidence. Furthermore, the integration of gene editing with innovative delivery systems, such as engineered bacteriophages, lipid nanoparticles, and viral vectors, underscores its adaptability across both ex vivo and in vivo therapeutic landscapes. The review also illuminates CRISPR's frontier role in developing next-generation diagnostics, offering unprecedented speed, specificity, and portability through platforms like SHERLOCK and DETECTR, especially vital in pandemic response and low-resource settings. Importantly, gene drives and synthetic biology applications in vector control—particularly against malaria and dengue—signal a paradigm shift in public health strategy, though accompanied by ethical, ecological, and regulatory complexities. Looking ahead, the synthesis of CRISPR with artificial intelligence, mRNA delivery platforms, and wearable diagnostics heralds a new era of personalized, programmable, and precision medicine. This article thus positions gene editing not merely as an ancillary tool but as a central pillar in the future architecture of infectious disease prevention, treatment, and global health resilience.

Keywords: CRISPR Therapeutics; Gene Editing; Infectious Diseases; Antiviral Strategies; Synthetic Biology; Genetic Diagnostics; Bacterial Resistance; Genome Engineering.

1. Introduction

Infectious diseases continue to pose a significant threat to global health, accounting for a substantial portion of morbidity and mortality worldwide. According to the Global Burden of Disease Study 2021, infectious diseases were

* Corresponding author: Fagbemi Oluwaseyi Ajibola; Email: seyikizz@gmail.com

responsible for approximately 28% of the total global disability-adjusted life years (DALYs), highlighting their persistent impact despite advancements in medical science [1].

Traditional therapeutic and diagnostic approaches have played pivotal roles in managing infectious diseases. However, these methods often face limitations, including the emergence of antimicrobial resistance, delayed diagnostic processes, and the inability to effectively target latent or persistent infections [2]. The increasing prevalence of drug-resistant pathogens further exacerbates these challenges, necessitating the exploration of innovative strategies to combat infectious agents [2,3].

Gene editing technologies have emerged as promising tools in the fight against infectious diseases. Techniques such as CRISPR-Cas systems offer the potential to precisely modify genetic material, enabling targeted disruption of viral genomes or enhancement of host immune responses . Recent studies have demonstrated the efficacy of gene editing in inhibiting the replication of viruses like HIV and hepatitis B, showcasing its therapeutic potential [4].

This review aims to provide a comprehensive overview of gene editing approaches in the context of infectious diseases. It will delve into the mechanisms of various gene editing technologies, their applications in therapeutic interventions and diagnostics, and the challenges associated with their implementation. By analyzing current research and clinical advancements, this article seeks to elucidate the role of gene editing in revolutionizing the management of infectious diseases.

2. Overview of Gene Editing Technologies

Table 1 Comparative Overview of Gene Editing Platforms for Infectious Disease Applications

Platform	Mechanism of Action	Target Type	Editing Precision	Delivery Mode	Key Applications	Reference
CRISPR-Cas9	sgRNA-guided double-strand break (DSB) at DNA target	DNA	High (subject to gRNA design)	AAV, electroporation, LNPs	HIV excision (LTRs), HBV cccDNA disruption	[6, 14–16]
CRISPR-Cas12a	T-rich PAM, staggered DSBs, only crRNA needed	DNA	High	AAV, nanoparticles	Multiplex gene editing, HPV	[11, 13]
CRISPR-Cas13	RNA-guided RNA cleavage with collateral activity	RNA	High (for RNA detection)	LNPs, SHERLOCK platform	SARS-CoV-2 RNA degradation	[12, 56, 57]
Base Editing	C-to-T or A-to-G base substitution without DSB	DNA	Very High	AAV, LNPs	β-thalassemia, SCD	[19, 23, 24]
Prime Editing	RT-mediated base-to-base conversion, insertion, deletion	DNA	Very High	Dual AAVs, LNPs	SCD, cystic fibrosis, β-thalassemia	[27, 35, 36]
TALENs	Protein-DNA binding causing DSBs	DNA	Moderate	Electroporation	HSV, HPV	[5]
ZFNs	Zinc finger DNA-binding causing DSBs	DNA	Low-Moderate	Viral vectors	HIV latency	[5]

Gene editing technologies have revolutionized the field of molecular biology, offering unprecedented precision in modifying genetic material. These tools have become instrumental in understanding gene functions, modeling diseases, and developing novel therapeutic strategies. This section provides a comprehensive overview of the major gene editing platforms, including CRISPR-Cas systems, base editing, prime editing, and earlier technologies such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [5]. To provide a foundational comparison of the diverse gene editing platforms discussed throughout this section, table 1 outlines their core mechanisms, delivery strategies, and applications relevant to infectious disease therapeutics and diagnostics. Also, to

contextualize the comparative capabilities of various gene editing platforms introduced in this section, Figure 1 provides a visual overview of ZFNs, TALENs, and CRISPR/Cas9 systems, highlighting differences in targeting mechanisms, editing precision, and ease of use.

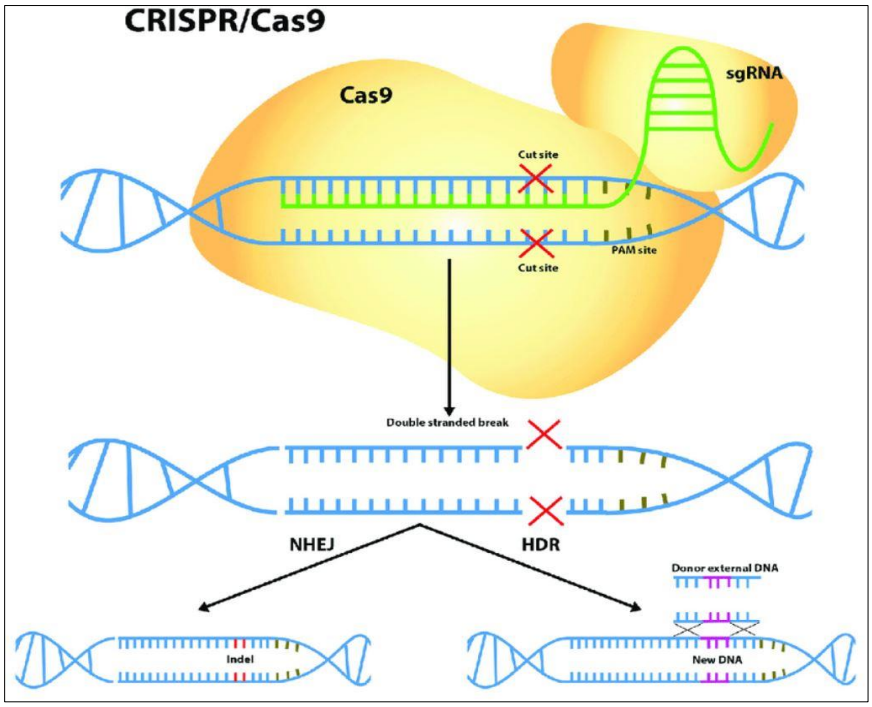

Figure 1 Comparison of Gene Editing Tools. A single guide RNA (sgRNA) binds to a specific genomic sequence adjacent to a 5'-NGG-3' protospacer adjacent motif (PAM), thereby directing the recruitment of the Cas9 DNA endonuclease. Reproduced with permission from Ref. [3].

2.1. CRISPR-Cas Systems

The CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats–CRISPR-associated proteins) system has revolutionized the field of genome editing due to its simplicity, efficiency, and versatility. Originally discovered as an adaptive immune mechanism in bacteria and archaea, this system allows for precise targeting and modification of genetic material. The most widely used variant, CRISPR-Cas9, employs a single-guide RNA (sgRNA) to direct the Cas9 nuclease to specific DNA sequences, facilitating targeted double-strand breaks (DSBs) and subsequent gene editing. This technology has been instrumental in advancing our understanding of gene function and holds significant promise for therapeutic applications [6].

2.1.1. Mechanism of CRISPR-Cas9

The CRISPR-Cas9 system operates through a multi-step process involving recognition, cleavage, and repair. The sgRNA, comprising a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA), guides the Cas9 nuclease to a complementary DNA sequence adjacent to a protospacer adjacent motif (PAM). Upon binding, Cas9 induces a DSB at the target site. The cell's endogenous repair mechanisms, primarily non-homologous end joining (NHEJ) or homology-directed repair (HDR), then resolve the break, leading to gene disruption or precise sequence modification, respectively [7,8].

The efficiency and specificity of CRISPR-Cas9-mediated editing are influenced by several factors, including sgRNA design, chromatin accessibility, and the choice of repair pathway. Advancements in sgRNA optimization have enhanced target specificity, reducing off-target effects. Moreover, the development of high-fidelity Cas9 variants, such as SpCas9-HF1 and eSpCas9, has further improved the precision of genome editing by minimizing unintended cleavage events [8]. The fundamental process of CRISPR/Cas9-mediated genome editing, including target recognition, double-strand break formation, and subsequent repair via non-homologous end joining or homology-directed repair, is visually summarized in Figure 2. This schematic facilitates understanding of the mechanistic underpinnings discussed above.

Despite these improvements, challenges remain in controlling the repair outcomes post-DSB induction. Strategies to bias repair towards HDR, such as cell cycle synchronization and the use of HDR-promoting small molecules, are being explored to enhance the accuracy of gene correction. Understanding and manipulating the cellular DNA repair machinery are critical for the successful application of CRISPR-Cas9 in therapeutic contexts [9,10].

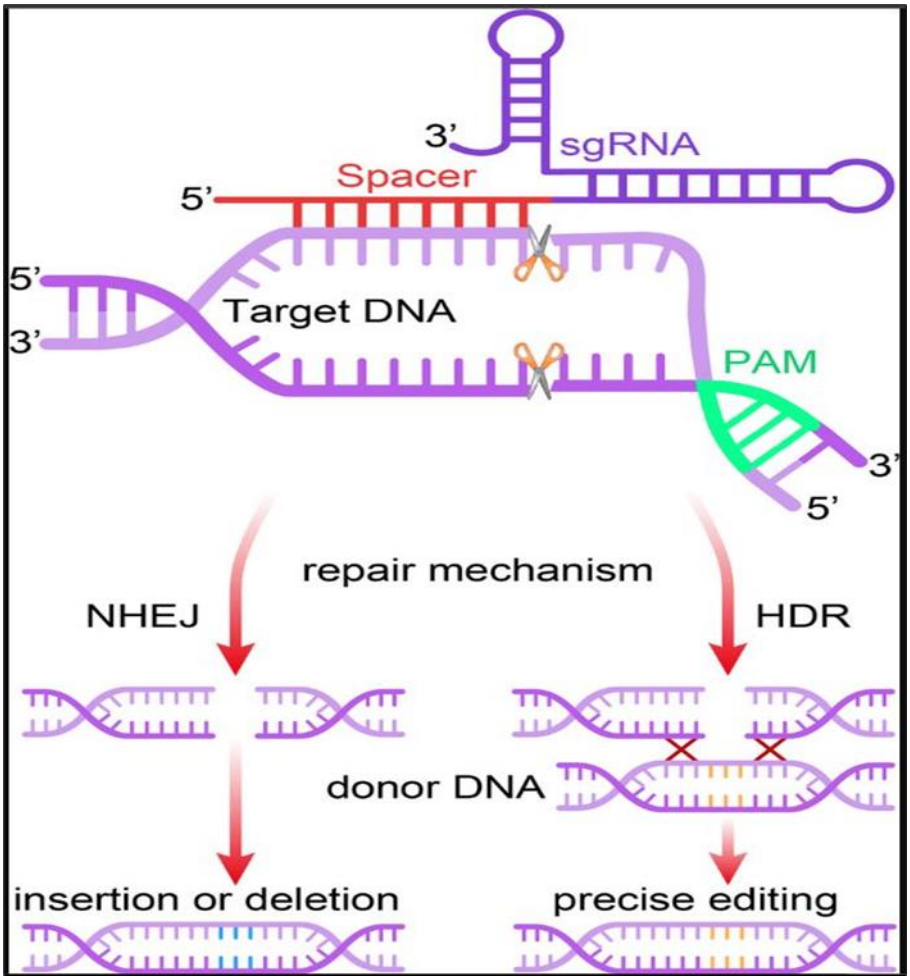

Figure 2 Mechanism of CRISPR/Cas9 Gene Editing. The single guide RNA (sgRNA) guides the Cas9 nuclease to a specific complementary sequence within the genome, where Cas9 introduces a double-strand break (DSB). For this process to occur, the target site must be immediately followed by a 5′-NGG-3′ protospacer adjacent motif (PAM). The resulting DSB can be repaired by either non-homologous end joining (NHEJ), which often leads to insertions or deletions, or by homology-directed repair (HDR) if a DNA repair template is provided—enabling precise genetic modifications or the insertion of exogenous sequences. Reproduced with permission from Ref. [9].

2.1.2. Variants of CRISPR-Cas Systems

Beyond Cas9, other Cas proteins have been identified, expanding the CRISPR toolkit. Cas12a (formerly Cpf1) is a Class 2, Type V effector that differs from Cas9 in several aspects: it recognizes a T-rich PAM, generates staggered DSBs with 5' overhangs, and requires only a crRNA for targeting. These features make Cas12a advantageous for certain applications, such as multiplexed gene editing and precise insertions [11]. Cas13, a Class 2, Type VI effector, uniquely targets RNA instead of DNA. Upon binding to its RNA target, Cas13 exhibits collateral RNase activity, cleaving nearby non-targeted RNAs. This property has been harnessed for sensitive and specific nucleic acid detection platforms, such as SHERLOCK and DETECTR, which have been applied in diagnostics for viral pathogens, including SARS-CoV-2 [12].

The discovery and characterization of these Cas variants have broadened the scope of CRISPR-based technologies. Ongoing research aims to identify and engineer new Cas proteins with diverse functionalities, improved specificity, and reduced off-target effects, thereby enhancing the versatility and safety of genome and transcriptome editing tools [13].

2.1.3. Applications in Infectious Diseases

CRISPR-Cas systems have shown significant potential in combating infectious diseases by enabling targeted disruption of pathogen genomes and modulation of host factors critical for infection. In the context of HIV-1, CRISPR-Cas9 has been employed to excise integrated proviral DNA from infected cells. Lai et al. [14] demonstrated that dual sgRNAs targeting the long terminal repeats (LTRs) of HIV-1 could effectively remove the provirus, leading to a reduction in viral replication in vitro.

Building upon these findings, Excision BioTherapeutics developed EBT-101, a CRISPR-based therapeutic designed to excise large portions of the HIV genome using dual sgRNAs delivered via adeno-associated virus (AAV) vectors. Preclinical studies in non-human primates showed promising results, with significant reductions in viral DNA levels. EBT-101 has progressed to Phase I/II clinical trials, marking a significant step towards a functional cure for HIV [15,16]. Table 2 presents a synthesis of notable viral pathogens targeted by CRISPR-based strategies, highlighting the specific viral regions edited, the delivery platforms used, and the resulting therapeutic outcomes as documented in both preclinical and clinical studies.

Beyond HIV, CRISPR-Cas systems have been explored for targeting other viral pathogens, such as hepatitis B virus (HBV) and human papillomavirus (HPV). By designing sgRNAs specific to viral genes essential for replication, researchers have achieved suppression of viral gene expression and replication in infected cells. These studies underscore the potential of CRISPR-based strategies as antiviral therapeutics, offering a novel approach to treating persistent viral infections [17,18]. Figure 3 visually encapsulates CRISPR/Cas9-based therapeutic strategies against key viral infections—HIV, HBV, and HPV—demonstrating specific genomic targets and molecular mechanisms utilized for viral suppression or genome excision.

Table 2 CRISPR-Based Therapeutic Strategies for Viral Infections

Virus	Target Region	Gene Editing Tool	Delivery Method	Editing Outcome	Clinical/Preclinical Evidence	Reference
HIV	LTRs, proviral DNA, CCR5	CRISPR-Cas9	AAV (EBT-101), electroporation	Provirus excision, host receptor disruption	Phase I/II (EBT-101), CCR5 trial (Zhang et al., 2025)	[14–16, 61]
HBV	cccDNA	CRISPR-Cas9, Base Editors	LNPs, AAV	Viral suppression, antigen reduction	Mouse/liver cell models	[17, 55, 58]
HSV	Latent DNA	TALENs, Cas9	Electroporation	Reactivation inhibition	In vitro studies	[5, 17]
HPV	E6/E7 oncogenes	Cas9, Cas12a	AAV, lipofection	Gene silencing, apoptosis	Cervical cell lines	[17, 11]
SARS-CoV-2	N, ORF1ab RNA regions	CRISPR-Cas13	LNPs, PAC-MAN, SHERLOCK	Viral RNA degradation	In vitro and diagnostic deployment	[12, 56–57]

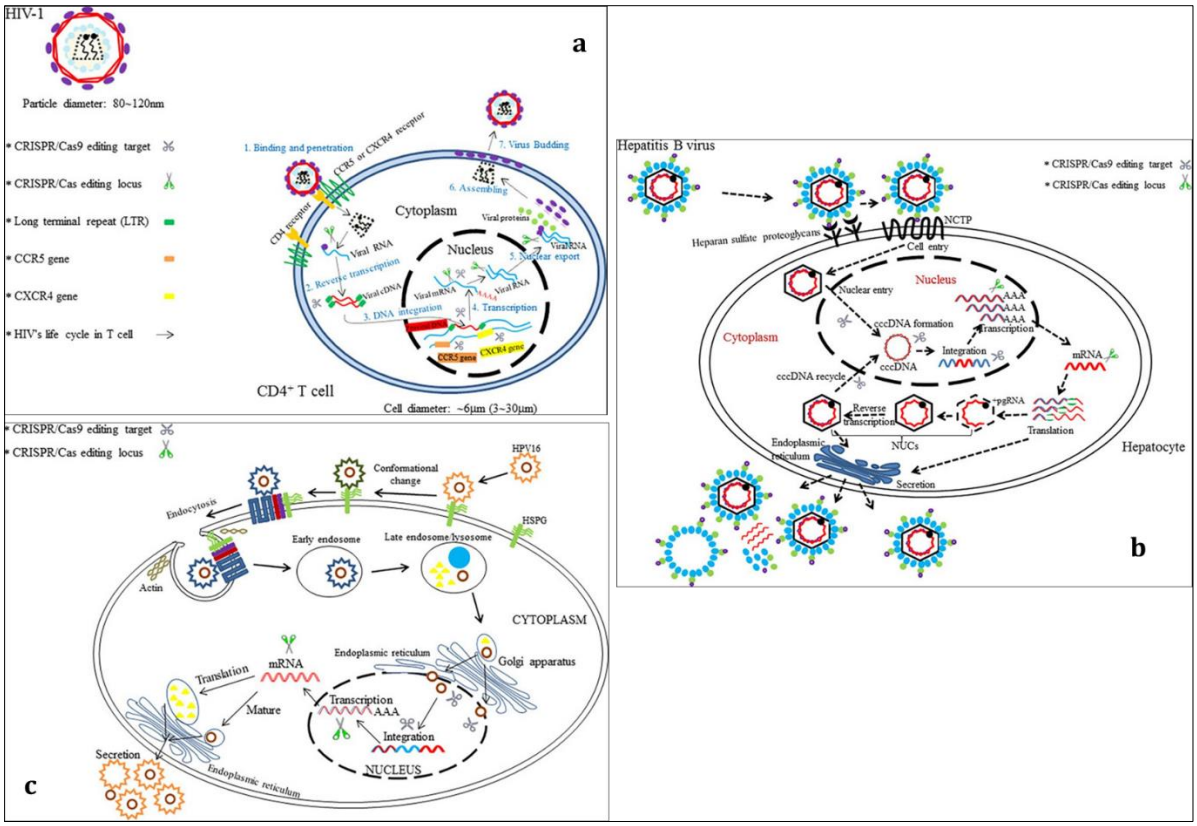

Figure 3 CRISPR/Cas9 Therapeutic Targets in HIV, HBV, and HPV, illustrating the application of CRISPR/Cas9 technology in targeting various stages of the life cycles of (a) HIV, (b) HBV, and (c) HPV. It highlights specific genomic regions of these viruses that are susceptible to CRISPR-mediated editing, offering insights into potential therapeutic interventions. Reproduced with permission from Ref. [18].

2.2. Base Editing

Base editing represents a significant advancement in genome engineering, enabling precise, irreversible conversion of specific DNA bases without introducing double-strand breaks (DSBs). This technology combines a catalytically impaired CRISPR-Cas9 (nickase) with a deaminase enzyme to directly convert one base into another at targeted genomic loci. By avoiding DSBs, base editing reduces the risk of unintended insertions or deletions (indels) and offers a more predictable editing outcome. The two primary classes of base editors are cytosine base editors (CBEs), which convert cytosine (C) to thymine (T), and adenine base editors (ABEs), which convert adenine (A) to guanine (G). These tools have expanded the scope of genetic modifications, offering therapeutic potential for various monogenic diseases [19]

2.2.1. Mechanism of Base Editors

Base editors function by coupling a DNA-targeting module, typically a Cas9 nickase guided by a single-guide RNA (sgRNA), with a deaminase enzyme that chemically modifies the target base. In CBEs, the deaminase converts cytosine to uracil, which is recognized as thymine during DNA replication, resulting in a C•G to T•A transition. ABEs, on the other hand, use an evolved tRNA adenosine deaminase to convert adenine to inosine, which is interpreted as guanine by the cellular machinery, leading to an A•T to G•C transition [20,21] The editing window, typically spanning positions 4 to 8 within the protospacer adjacent motif (PAM)-proximal region, determines the efficiency and specificity of base editing. Precise positioning of the target base within this window is crucial to achieve the desired edit while minimizing bystander effects. Advancements in sgRNA design and engineering of deaminase domains have improved the targeting scope and reduced off-target activities [22].

Despite these improvements, base editing is limited to transition mutations and cannot induce transversions or larger genomic alterations. Furthermore, the requirement for specific PAM sequences restricts the targetable regions within the genome. Ongoing research aims to develop novel Cas variants with relaxed PAM requirements and expand the editing capabilities of base editors.

2.2.2. Therapeutic Applications

Base editing has shown promise in correcting pathogenic point mutations responsible for various genetic disorders. For instance, researchers have utilized ABEs to correct the IVS1-110 (G>A) mutation in the β-globin gene, a common cause of β-thalassemia. In vitro and in vivo studies demonstrated efficient correction of the mutation in patient-derived hematopoietic stem and progenitor cells (HSPCs), leading to restored hemoglobin production [23,24]. Similarly, base editing has been applied to treat sickle cell disease (SCD) by inducing mutations that reactivate fetal hemoglobin (HbF) expression. By targeting regulatory elements of the BCL11A gene, base editors can disrupt its expression, thereby increasing HbF levels and ameliorating the sickling phenotype. Preclinical studies have shown that this approach effectively reduces disease symptoms in animal models [24].

Beyond hematological disorders, base editing holds potential for treating other monogenic diseases, such as hereditary tyrosinemia, phenylketonuria, and certain forms of muscular dystrophy. The ability to precisely correct point mutations without introducing DSBs makes base editing an attractive strategy for therapeutic genome modification.

2.2.3. Limitations and Challenges

Despite its advantages, base editing faces several limitations that need to be addressed for clinical applications. One major concern is the occurrence of off-target edits, both at DNA and RNA levels, which can lead to unintended mutations and potential safety risks. Efforts to engineer high-fidelity deaminase variants and optimize delivery methods are ongoing to minimize these off-target effects [25].

Another challenge is the limited editing window and sequence constraints imposed by the requirement for specific PAM sequences. This restricts the range of targetable sites within the genome. To overcome this, researchers are developing base editors with expanded PAM compatibility and broader editing windows, enhancing their versatility. Furthermore, efficient delivery of base editors to target cells and tissues remains a hurdle. Viral vectors, such as adeno-associated viruses (AAVs), have been commonly used but are limited by packaging capacity and potential immunogenicity. Non-viral delivery methods, including lipid nanoparticles and electroporation of ribonucleoprotein complexes, are being explored to improve delivery efficiency and safety [26].

2.3. Prime Editing

Prime editing is a versatile and precise genome-editing technology that enables targeted insertions, deletions, and all 12 possible base-to-base conversions without requiring double-strand breaks (DSBs) or donor DNA templates. This method combines a catalytically impaired Cas9 nickase fused to a reverse transcriptase (RT) enzyme, guided by a prime editing guide RNA (pegRNA) that specifies the target site and encodes the desired edit. Since its introduction by Anzalone et al. [27] in 2019, prime editing has undergone significant advancements, enhancing its efficiency, specificity, and therapeutic potential. This section delves into the mechanism of prime editing, its therapeutic applications, and the challenges that need to be addressed for its clinical translation [28,29].

2.3.1. Mechanism of Prime Editors

Prime editing operates through a "search-and-replace" mechanism, wherein the Cas9 nickase-RT fusion protein is directed to a specific genomic locus by the pegRNA (See Figiure 4). The pegRNA comprises a spacer sequence that guides the complex to the target site and a primer binding site (PBS) followed by a reverse transcription template (RTT) that encodes the desired edit. Upon binding, the Cas9 nickase induces a single-strand break in the DNA, allowing the RT to synthesize the edited sequence using the RTT as a template. The newly synthesized strand incorporates the intended modification, which is then resolved by the cell's DNA repair machinery to complete the editing process [30].

According to Anzalone et al. [31], the initial prime editing system, termed PE1, demonstrated the feasibility of this approach but exhibited modest editing efficiencies. Subsequent iterations, such as PE2 and PE3, introduced enhancements like engineered RTs with improved activity and additional nicking of the non-edited strand to bias repair towards the edited sequence, thereby increasing editing efficiency and product purity. Further developments, including PE4 and PE5, incorporated mismatch repair inhibitors to suppress undesired repair pathways, leading to even higher editing efficiencies and reduced byproducts. These advancements have expanded the applicability of prime editing across various cell types and genomic contexts [29,32].

Recent studies have also explored the structural optimization of pegRNAs to enhance stability and functionality. For instance, Nelson et al. [33] identified the small RNA-binding protein La as a key factor in promoting prime editing efficiency. By fusing the N-terminal domain of La to the prime editor, they developed PE7, which exhibited improved

editing outcomes across multiple systems. This innovation underscores the importance of pegRNA design and protein engineering in refining prime editing technologies. [32,33].

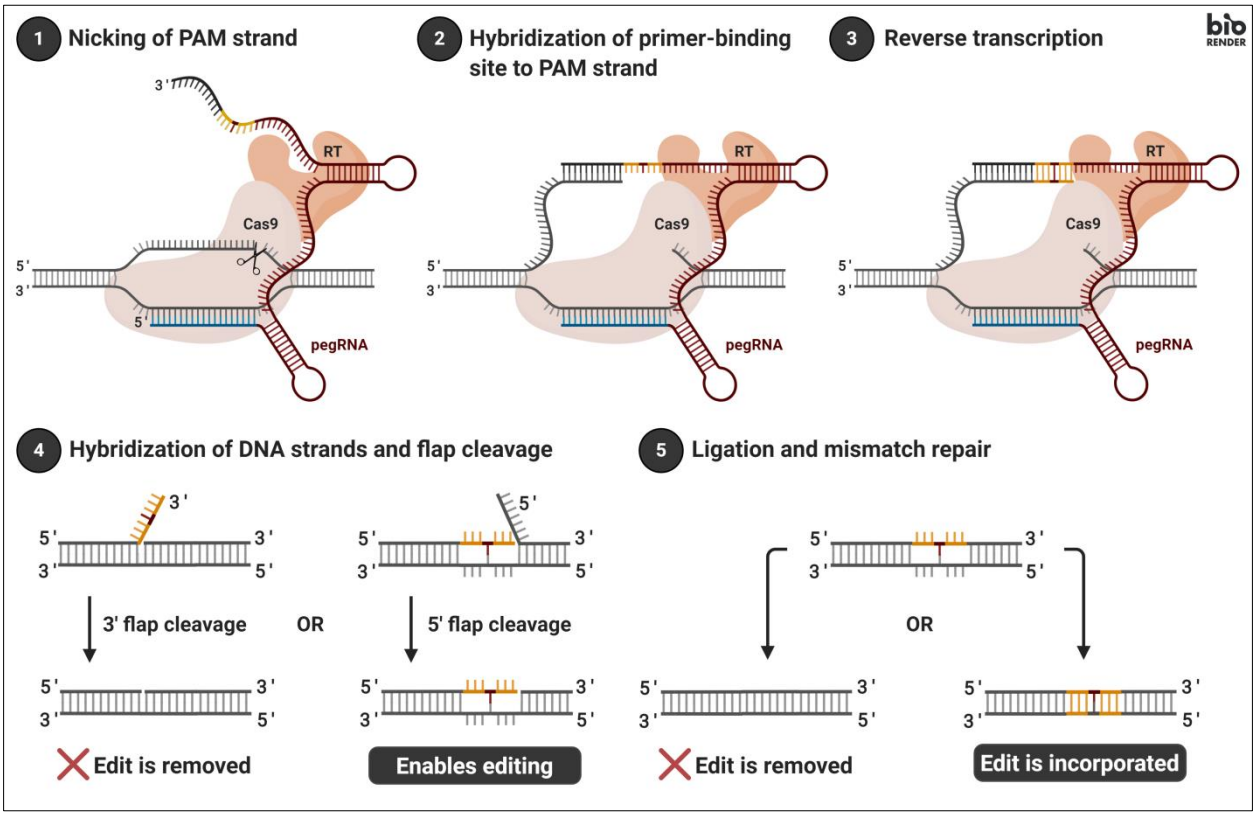

Figure 4 Mechanism of Prime Editing. Schematic representation of the prime editing process, which utilizes a Cas9 nickase fused to a reverse transcriptase (RT) and guided by a prime editing guide RNA (pegRNA). By Ldinatto - Own work, CC BY-SA 4.0, from Ref. [33].

2.3.2. Therapeutic Applications

The precision and versatility of prime editing make it a promising tool for correcting pathogenic mutations underlying various genetic disorders. Unlike traditional CRISPR-Cas9 approaches that rely on DSBs and homology-directed repair, prime editing minimizes the risk of unintended insertions or deletions, offering a safer alternative for therapeutic genome modification [34]. In the context of hematological diseases, prime editing has been employed to correct mutations responsible for conditions like sickle cell disease and β-thalassemia. For example, Everette et al. [35] demonstrated the efficient correction of the β-globin gene mutation in patient-derived hematopoietic stem and progenitor cells using prime editing, leading to restored hemoglobin production and amelioration of disease phenotypes. Similarly, prime editing has been utilized to introduce protective mutations that induce fetal hemoglobin expression, offering therapeutic benefits for sickle cell disease patients.

Beyond hematological disorders, prime editing holds potential for treating a wide range of monogenic diseases, including cystic fibrosis, Tay-Sachs disease, and certain forms of muscular dystrophy. In a notable study, Sousa et al. [36] applied prime editing to correct the ΔF508 mutation in the CFTR gene, which is the most common cause of cystic fibrosis. The edited cells exhibited restored CFTR function, highlighting the therapeutic promise of prime editing for this debilitating condition.

Moreover, prime editing has been explored for in vivo applications, with researchers developing delivery strategies using adeno-associated viruses (AAVs) and lipid nanoparticles to target specific tissues. These approaches have shown success in animal models, paving the way for future clinical trials aimed at treating genetic diseases directly within the patient's body.

2.3.3. Limitations and Challenges

Despite its advantages, prime editing faces several challenges that must be addressed to facilitate its clinical translation. One significant hurdle is the relatively large size of the prime editor construct, which complicates delivery using commonly employed vectors like AAVs. To overcome this, researchers have been exploring alternative delivery methods, including dual-AAV systems and non-viral approaches such as lipid nanoparticles, to efficiently transport the prime editor components into target cells [37-39].

Another concern is the variability in editing efficiency across different cell types and genomic loci. Factors such as chromatin accessibility, DNA repair pathway activity, and pegRNA design can influence the success of prime editing. Efforts to optimize pegRNA structures, including modifications to the PBS and RTT regions, have shown promise in enhancing editing outcomes. Additionally, the development of computational tools to predict optimal pegRNA designs based on target site characteristics is underway to improve the reliability of prime editing [38].

Off-target effects remain a critical consideration for any genome-editing technology. While prime editing is generally associated with fewer off-target mutations compared to traditional CRISPR-Cas9 systems, comprehensive assessments are necessary to ensure its safety for therapeutic applications. High-throughput sequencing and unbiased genome-wide analyses are being employed to evaluate the specificity of prime editing and identify potential unintended edits. Continued refinement of the prime editor components and delivery methods is essential to minimize off-target effects and enhance the precision of this technology [37,38].

2.4. Delivery Strategies for CRISPR-Based Genome Editing

The efficacy of CRISPR-based genome editing is intrinsically linked to the development of efficient and safe delivery systems that can transport the editing machinery to target cells and tissues [40]. Delivery strategies are broadly categorized into ex vivo and in vivo approaches, each with distinct methodologies, advantages, and challenges [41]. This section provides a comprehensive analysis of these delivery strategies, highlighting recent advancements and ongoing challenges in the field.

2.4.1. Ex Vivo Delivery Strategies

Ex vivo delivery involves the extraction of cells from a patient, their genetic modification outside the body, and subsequent reintroduction into the patient. This approach offers precise control over the editing process and allows for thorough screening of modified cells before transplantation. According to Li et al. [42], ex vivo strategies have been successfully employed in clinical settings, particularly for hematopoietic stem cell modifications aimed at treating blood disorders such as sickle cell disease and β-thalassemia. The ex vivo method typically utilizes electroporation to introduce CRISPR components into cells. This technique transiently permeabilizes the cell membrane, allowing the entry of ribonucleoprotein complexes. Studies by Dever et al. [43] have demonstrated high editing efficiencies using electroporation, with minimal off-target effects, in primary human T cells. Additionally, the ex vivo approach facilitates the use of selection markers and expansion of edited cells, ensuring a homogeneous population for therapeutic applications [43,44].

However, ex vivo delivery is not without limitations. The process is labor-intensive, requires specialized facilities, and may not be suitable for all cell types. Furthermore, the manipulation of cells outside their native environment can affect their functionality and viability. Efforts are ongoing to streamline ex vivo protocols and develop automated systems to enhance scalability and reproducibility [44,45].

2.4.2. In Vivo Delivery Strategies

In vivo delivery entails the direct administration of CRISPR components into the patient's body, targeting specific tissues or organs. This approach is particularly advantageous for treating diseases where ex vivo manipulation is impractical [46]. According to Lino et al. [47], viral vectors, such as adeno-associated viruses (AAVs), have been extensively used for in vivo delivery due to their high transduction efficiency and tissue specificity. For instance, AAV-mediated delivery of CRISPR components has shown promise in correcting genetic mutations in animal models of muscular dystrophy and retinal diseases [44].

Non-viral delivery methods are also being explored to overcome the limitations associated with viral vectors, such as immunogenicity and limited cargo capacity. Lipidoid nanoparticles (LNPs) have emerged as a promising alternative, capable of encapsulating and delivering CRISPR components efficiently. Research by Finn et al. [48] demonstrated successful in vivo genome editing in mouse models using LNPs, highlighting their potential for clinical applications. The diversity and functionality of delivery platforms—critical to the success of in vivo gene editing—are illustrated in Figure

Table 3 Delivery Systems for CRISPR-Based Therapeutics

Delivery System	Type	Advantages	Limitations	Disease Targets	Reference
Adeno-Associated Virus (AAV)	Viral	High transduction efficiency, tissue-specific	Size limit (~4.7 kb), immune response	HIV (EBT-101), HBV	[16, 47]
Lentivirus	Viral	Integrates into dividing/non-dividing cells	Insertional mutagenesis, safety concerns	Ex vivo T-cell editing for HIV	[42, 44]
Lipid Nanoparticles (LNPs)	Non-viral	Low immunogenicity, scalable	Tissue targeting limitation	SARS-CoV-2, in vivo liver editing	[48, 58]
Electroporation	Physical	High efficiency in HSPCs, no vector	Can reduce cell viability	Ex vivo editing of hematopoietic stem cells	[43, 45]
mRNA Platforms	Synthetic	Transient, scalable, safe	Delivery & stability concerns	Cas9 protein delivery, diagnostics	[114–116]

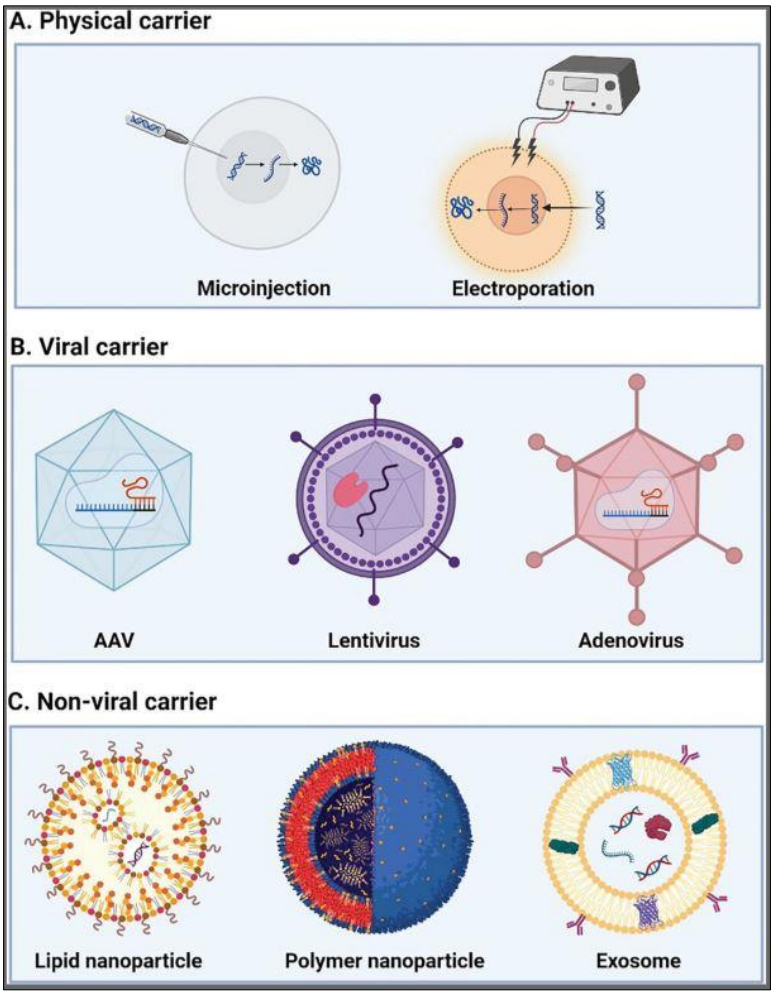

Figure 5 CRISPR Delivery Systems presenting various CRISPR delivery methods, including physical (microinjection, electroporation), viral (AAV, lentivirus), and non-viral (lipid nanoparticles, exosomes) systems, along with their respective advantages and limitations. Reproduced with permission from Ref. [45].

This diagram outlines viral and non-viral vectors, detailing their advantages and inherent limitations in the context of therapeutic delivery.

As can be rightly deduced from the foregoing, the success of CRISPR-mediated interventions relies heavily on the development of efficient delivery platforms. Table 3 provides a comparative overview of commonly used viral and non-viral delivery systems, highlighting their advantages, limitations, and applications in the context of infectious disease treatment.

2.4.3. Challenges and Future Directions

The delivery of CRISPR components remains a critical bottleneck in the translation of genome editing technologies to clinical therapies. One of the primary challenges is achieving efficient and specific delivery to target cells while minimizing off-target effects and immune responses. According to Wang et al. [49], the development of novel delivery systems that combine the advantages of both viral and non-viral methods is a promising avenue for overcoming these obstacles.

Another significant concern is the potential for unintended genomic alterations resulting from prolonged expression of CRISPR components. To mitigate this risk, researchers are exploring transient delivery methods and inducible systems that allow for controlled expression of the editing machinery. For example, Wang et al. [50] developed a self-limiting CRISPR system that reduces off-target effects by restricting the duration of Cas9 activity.

Furthermore, the scalability and manufacturability of delivery systems are essential considerations for clinical translation. Efforts are being made to develop standardized protocols and scalable production methods to facilitate the widespread adoption of CRISPR-based therapies [51]. Collaborative initiatives between academia, industry, and regulatory agencies are crucial to address these challenges and advance the field of genome editing. A comparative understanding of in vivo and ex vivo delivery strategies is essential for tailoring gene editing interventions to disease context. Table 4 outlines the distinguishing features, benefits, and limitations of these approaches, using illustrative examples from current gene editing therapies for infectious diseases.

Table 4 In Vivo vs. Ex Vivo Gene Editing for Infectious Disease Therapies

Feature	In Vivo Approach	Ex Vivo Approach	Reference
Description	Direct gene editing within the patient's body	Editing in isolated patient cells, then reinfusion	[46, 59]
Delivery Method	AAV, LNP, mRNA	Electroporation, lentiviral transduction	[44, 47]
Control Over Editing	Limited	High (pre-screening possible)	[42, 43]
Immune Risk	Elevated due to systemic exposure	Lower	[46]
Examples	HBV cccDNA targeting in hepatocytes	CCR5 editing in CD4+ T cells for HIV	[59, 61]
Limitations	Delivery specificity, immune clearance	Cost, scalability	[44, 45]

3. CRISPR-Based Antiviral Therapeutics

The advent of CRISPR-based genome editing has revolutionized the landscape of antiviral therapeutics. By enabling precise targeting and modification of viral genomes, CRISPR technologies offer promising avenues for the treatment of various viral infections. This section delves into the mechanistic targeting of viral genomes, explores both in vivo and ex vivo applications, reviews preclinical and clinical trial outcomes, and discusses the challenges associated with viral escape, delivery, and immunogenicity.

3.1. Mechanistic Targeting of Viral Genomes

CRISPR-based systems, particularly CRISPR/Cas9 and CRISPR/Cas13, have been harnessed to target and disrupt viral genomes, thereby inhibiting viral replication and propagation. These systems utilize guide RNAs to direct the Cas nucleases to specific sequences within the viral genome, leading to targeted cleavage and subsequent degradation or mutation of viral DNA or RNA. This approach has been applied to a range of viruses, including HIV, HBV, HSV, and SARS-CoV-2 [52,53].

In the context of HIV, CRISPR/Cas9 has been employed to excise proviral DNA from infected host genomes, effectively reducing viral load and preventing reactivation. Studies have demonstrated the successful targeting of long terminal repeats (LTRs) and essential viral genes, leading to the inactivation of latent HIV reservoirs [54]. Similarly, for HBV, CRISPR/Cas9 has been utilized to target and disrupt covalently closed circular DNA (cccDNA), a persistent form of the viral genome responsible for chronic infection [55]. This strategy has shown promise in reducing HBV replication and antigen expression.

For RNA viruses like SARS-CoV-2, CRISPR/Cas13 systems have been developed to target and degrade viral RNA genomes. The PAC-MAN (Prophylactic Antiviral CRISPR in huMAN cells) approach employs Cas13d to cleave conserved regions of the SARS-CoV-2 genome, thereby inhibiting viral replication. This method has demonstrated efficacy in vitro, highlighting the potential of CRISPR-based strategies against emerging RNA viruses [56,57].

3.2. In Vivo and Ex Vivo Applications

CRISPR-based antiviral therapeutics can be administered through in vivo or ex vivo approaches, each with distinct methodologies and applications. In vivo applications involve the direct delivery of CRISPR components into the patient's body to target infected cells [58]. This method has been explored for treating viral infections such as HBV and SARS-CoV-2. For instance, lipid nanoparticle-mediated delivery of CRISPR/Cas9 has been investigated for targeting HBV cccDNA in hepatocytes, demonstrating significant reductions in viral markers in preclinical models. Similarly, in vivo delivery of CRISPR/Cas13 systems has been proposed for targeting SARS-CoV-2 RNA in respiratory epithelial cells [58].

Ex vivo applications entail the extraction of patient-derived cells, their genetic modification using CRISPR systems outside the body, and subsequent reinfusion [59]. This approach has been particularly effective in the context of HIV, where ex vivo editing of hematopoietic stem cells to disrupt the CCR5 co-receptor gene has conferred resistance to HIV infection. Clinical trials have demonstrated the feasibility and safety of this strategy, with edited cells engrafting successfully and providing durable protection against HIV [59].

Both in vivo and ex vivo approaches offer unique advantages and challenges. In vivo methods are less invasive and can target tissues that are difficult to access ex vivo, but they face hurdles related to delivery efficiency and off-target effects. Ex vivo techniques allow for precise editing and rigorous screening of modified cells but involve complex procedures and are limited to certain cell types.

3.3. Preclinical and Clinical Trial Outcomes

Preclinical studies have provided robust evidence supporting the efficacy of CRISPR-based antiviral therapies. In animal models of HIV, CRISPR/Cas9-mediated excision of proviral DNA has led to sustained suppression of viral replication and a reduction in latent reservoirs. Similarly, in HBV-infected models, targeting cccDNA with CRISPR/Cas9 has resulted in decreased viral load and antigen levels. For SARS-CoV-2, in vitro studies using CRISPR/Cas13 have demonstrated effective degradation of viral RNA, paving the way for potential therapeutic applications [56,60].

Clinical trials have begun to translate these findings into human applications. A notable example is the ongoing trial by Excision BioTherapeutics, which employs CRISPR/Cas9 to excise HIV proviral DNA from infected individuals. Preliminary results indicate a favorable safety profile and potential efficacy in reducing viral reservoirs. Additionally, trials targeting the CCR5 gene in hematopoietic stem cells have shown promise in conferring resistance to HIV infection, with edited cells demonstrating long-term engraftment and functionality [61].

While these early clinical outcomes are encouraging, further studies are necessary to assess long-term safety, efficacy, and potential off-target effects. Continued monitoring and optimization of delivery methods, editing efficiency, and immune responses will be critical for the successful translation of CRISPR-based antiviral therapies into widespread clinical use.

3.4. Challenges of Viral Escape, Delivery, and Immunogenicity

Despite the promising potential of CRISPR-based antiviral therapeutics, several challenges must be addressed to ensure their safe and effective application. One significant concern is the possibility of viral escape through mutations in target sequences, rendering CRISPR systems ineffective. Viruses, particularly RNA viruses like HIV and SARS-CoV-2, exhibit high mutation rates, which can lead to the emergence of resistant strains [62]. To mitigate this risk, strategies such as multiplexed targeting of conserved viral regions and combination therapies are being explored to enhance the robustness of CRISPR-based interventions.

Efficient and targeted delivery of CRISPR components remains a critical hurdle, especially for in vivo applications. Delivery vectors must navigate biological barriers, achieve specific targeting of infected cells, and minimize off-target effects. Various delivery systems, including viral vectors like adeno-associated viruses (AAVs) and non-viral methods such as lipid nanoparticles, are under investigation to optimize delivery efficiency and safety [62].

4. Gene Editing Strategies for Bacterial Infections

The escalating crisis of antibiotic resistance has necessitated the exploration of innovative therapeutic strategies. Among these, CRISPR-based gene editing has emerged as a promising approach to combat bacterial infections. By leveraging the precision of CRISPR systems, researchers aim to develop targeted interventions that can either disrupt resistance mechanisms or directly eliminate pathogenic bacteria. This section delves into the application of CRISPR technology in engineering bacteriophages to target antibiotic-resistant bacteria, highlighting the methodologies, challenges, and advancements in this domain.

4.1. Engineering Bacteriophages with CRISPR to Target Antibiotic-Resistant Bacteria

Bacteriophages, viruses that specifically infect bacteria, have been revisited as potential therapeutic agents against multidrug-resistant bacterial strains. The integration of CRISPR-Cas systems into bacteriophages has enhanced their specificity and efficacy, offering a dual mechanism of action: the natural lytic activity of phages and the gene-editing capabilities of CRISPR. This synergy allows for the precise targeting and elimination of antibiotic resistance genes within bacterial populations.

4.1.1. CRISPR-Cas-Enhanced Phage Therapy

The incorporation of CRISPR-Cas systems into bacteriophages has led to the development of CRISPR-enhanced phage therapy. This approach involves engineering phages to deliver CRISPR-Cas components into bacterial cells, enabling the targeted cleavage of resistance genes or essential bacterial genes, leading to cell death. For instance, Locus Biosciences has developed a CRISPR-Cas3-enhanced bacteriophage cocktail, LBP-EC01, targeting Escherichia coli responsible for urinary tract infections. In a Phase 1b clinical trial, LBP-EC01 demonstrated safety and efficacy, with a significant reduction in bacterial load observed in patients [63].

Similarly, the SNIPR001 phage cocktail, engineered to target E. coli in biofilms, has shown superior efficacy compared to its individual components. In mouse models, SNIPR001 reduced E. coli load in the gut more effectively than traditional phage therapy, indicating the potential of CRISPR-enhanced phages in treating complex infections [64].

4.1.2. Strategies for Phage Engineering

Engineering bacteriophages with CRISPR systems involves several strategies to enhance their therapeutic potential. One approach is the modification of phage genomes to carry CRISPR-Cas constructs that target specific bacterial genes. This requires precise genome editing techniques to ensure the stability and functionality of the inserted CRISPR components. Additionally, phages can be engineered to broaden their host range, allowing them to infect a wider array of bacterial strains. This is achieved by altering phage receptor-binding proteins to recognize different bacterial surface molecules [65].

Another strategy involves the use of anti-CRISPR proteins to regulate the activity of CRISPR systems within phages. By incorporating anti-CRISPR genes, researchers can fine-tune the timing and extent of CRISPR-mediated gene editing, minimizing potential off-target effects and enhancing safety. These engineering strategies aim to create bacteriophages that are not only effective in eliminating antibiotic-resistant bacteria but also safe and adaptable for clinical applications [65,66].

4.2. Disruption of Resistance Genes in Pathogenic Bacteria

The escalating threat of antimicrobial resistance (AMR) has necessitated the exploration of innovative strategies to combat pathogenic bacteria. Among these, the utilization of CRISPR-Cas systems to disrupt resistance genes offers a promising avenue. This section delves into the methodologies, applications, and challenges associated with employing CRISPR technology to target and eliminate resistance determinants in pathogenic bacteria.

4.2.1. Targeting Plasmid-Borne Resistance Genes

Plasmids play a pivotal role in the dissemination of antibiotic resistance genes among bacterial populations. CRISPR-Cas systems have been harnessed to specifically target and eliminate these plasmid-borne resistance genes, thereby re-sensitizing bacteria to antibiotics [67].

For instance, studies have demonstrated the successful application of CRISPR-Cas9 systems to target the mcr-1 gene, which confers resistance to colistin, a last-resort antibiotic. By designing guide RNAs (gRNAs) specific to the mcr-1 gene, researchers were able to selectively cleave and eliminate the plasmids harboring this gene in Escherichia coli strains. This intervention not only eradicated the resistance gene but also restored the susceptibility of the bacteria to colistin [67].

Similarly, CRISPR-Cas systems have been employed to target other plasmid-encoded resistance genes, such as bla_TEM-1 and bla_NDM-1, which are responsible for β-lactam and carbapenem resistance, respectively. By eliminating these plasmids, the bacterial strains exhibited increased sensitivity to the corresponding antibiotics, highlighting the potential of CRISPR-mediated plasmid curing as a therapeutic strategy [67]. To contextualize the application of CRISPR tools in countering bacterial resistance, Table 5 summarizes key studies demonstrating the use of gene editing in different bacterial pathogens, with a focus on the targeted resistance mechanisms, delivery modalities, and observed therapeutic benefits.

Table 5 CRISPR Applications in Combating Antibiotic-Resistant Bacteria

Bacterial Pathogen	Resistance Mechanism	Gene Editing Target	Tool Used	Delivery Strategy	Outcome	Reference
Escherichia coli	Colistin resistance via mcr-1 plasmid	mcr-1 gene	CRISPR-Cas9	Plasmid-encoded phagemid	Resensitization to colistin	[67]
Staphylococcus aureus (MRSA)	mecA gene encoding PBP2a	mecA	Cas9, CRISPRi	Phagemid, engineered phages	Restored β-lactam sensitivity	[68, 81]
Mycobacterium tuberculosis	Intrinsic resistance, complex genome	Various virulence and resistance genes	Endogenous Cas10, CRISPR-Cas9	CRISPRi vectors	Gene repression, enhanced diagnostics	[78–80]

4.2.2. Chromosomal Resistance Gene Disruption

While plasmid-borne resistance genes are significant contributors to AMR, chromosomal mutations also play a crucial role. CRISPR-Cas systems offer the capability to target and disrupt these chromosomal resistance determinants [68]. In Staphylococcus aureus, the mecA gene, located on the chromosome, confers resistance to methicillin. By designing CRISPR-Cas9 systems targeting mecA, researchers achieved a significant reduction in gene expression, leading to increased susceptibility of the bacteria to β-lactam antibiotics [68].

Moreover, CRISPR-based approaches have been utilized to target other chromosomal genes associated with resistance, such as gyrA in Enterohemorrhagic E. coli (EHEC). Targeting these genes resulted in the attenuation of bacterial virulence and enhanced antibiotic susceptibility [69].

4.2.3. Prevention of Horizontal Gene Transfer

Horizontal gene transfer (HGT) is a primary mechanism through which bacteria acquire resistance genes. CRISPR-Cas systems can be engineered to prevent the acquisition of resistance genes via HGT, thereby curbing the spread of AMR [70]. In a notable study, Lee and coleagues [70] developed a CRISPR-Cas9 system that protected Escherichia coli from acquiring resistance genes through transformation, transduction, and conjugation. By targeting multiple resistance genes, the system effectively blocked the uptake of these genes, demonstrating a significant reduction in HGT events. Furthermore, the integration of CRISPR systems into probiotic strains has been explored to prevent the acquisition and dissemination of resistance genes within the gut microbiota. This approach holds promise in maintaining the efficacy of probiotics while mitigating the risk of AMR spread [70].

4.2.4. Challenges and Considerations

Despite the promising potential of CRISPR-based strategies in disrupting resistance genes, several challenges persist. The delivery of CRISPR components into bacterial populations, especially in vivo, remains a significant hurdle. Various delivery methods, including phagemids and conjugative plasmids, are being explored to enhance efficiency and specificity .

Additionally, the emergence of anti-CRISPR proteins encoded by bacteria poses a threat to the efficacy of CRISPR interventions. These proteins can inhibit CRISPR-Cas activity, necessitating the development of strategies to circumvent or neutralize their effects [68]. Moreover, the potential off-target effects and unintended consequences of CRISPR-mediated gene editing underscore the need for rigorous validation and safety assessments before clinical application.

4.3. CRISPR Antimicrobials vs. Traditional Antibiotics

The escalating threat of antimicrobial resistance (AMR) has necessitated the exploration of innovative therapeutic strategies. Among these, CRISPR-based antimicrobials have emerged as a promising alternative to traditional antibiotics. This section delves into the comparative analysis of CRISPR antimicrobials and conventional antibiotics, focusing on their mechanisms, efficacy, specificity, and potential in combating resistant bacterial infections.

4.3.1. Mechanisms of Action

Traditional Antibiotics:

Traditional antibiotics function by targeting essential bacterial processes, such as cell wall synthesis (e.g., β-lactams), protein synthesis (e.g., aminoglycosides), nucleic acid synthesis (e.g., fluoroquinolones), and metabolic pathways (e.g., sulfonamides). These agents often exhibit broad-spectrum activity, affecting both pathogenic and commensal bacteria. However, the overuse and misuse of antibiotics have led to the emergence of resistant strains, diminishing their efficacy [71].

CRISPR Antimicrobials:

CRISPR-based antimicrobials employ the CRISPR-Cas system to target and cleave specific DNA sequences within bacterial genomes or plasmids. By designing guide RNAs (gRNAs) complementary to resistance genes or virulence factors, CRISPR-Cas systems can selectively disrupt these elements, leading to bacterial cell death or resensitization to antibiotics. This precision reduces off-target effects and preserves beneficial microbiota. Moreover, CRISPR systems can be delivered via bacteriophages, plasmids, or nanoparticles, enhancing their versatility in therapeutic applications [72,73].

4.3.2. Efficacy and Specificity

Efficacy:

CRISPR antimicrobials have demonstrated high efficacy in preclinical studies. For instance, Locus Biosciences developed a CRISPR-Cas3-enhanced bacteriophage cocktail targeting Escherichia coli responsible for urinary tract infections. In a Phase 1b clinical trial, the treatment significantly reduced bacterial load in patients, showcasing its potential as an effective antimicrobial therapy [74].

Specificity:

One of the notable advantages of CRISPR antimicrobials is their high specificity. By designing gRNAs to target unique sequences within pathogenic bacteria, CRISPR systems can minimize collateral damage to the host's beneficial microbiota. This contrasts with broad-spectrum antibiotics, which often disrupt the balance of the microbiome, leading to secondary infections or dysbiosis [75,76].

4.3.3. Resistance Development and Mitigation

Traditional Antibiotics:

The widespread use of antibiotics has led to the rapid emergence of resistant bacterial strains through mechanisms such as gene mutation, horizontal gene transfer, and biofilm formation. These adaptations have rendered many antibiotics ineffective, posing significant challenges in treating infections [71].

CRISPR Antimicrobials:

CRISPR-based approaches offer a strategic advantage in mitigating resistance development. By targeting and disrupting resistance genes directly, CRISPR systems can eliminate the genetic basis for resistance. Furthermore, the adaptability of CRISPR allows for the rapid redesign of gRNAs to counteract emerging resistance mechanisms. However, challenges such as the potential for off-target effects and the need for efficient delivery systems remain areas of ongoing research [77].

4.4. Case Studies in Mycobacterium tuberculosis, Escherichia coli, and Staphylococcus aureus

The application of CRISPR-Cas systems in combating bacterial infections has garnered significant attention, particularly in addressing antibiotic resistance and enhancing diagnostic capabilities. This section delves into detailed case studies focusing on *Mycobacterium tuberculosis* (Mtb), *Escherichia coli* (E. coli), and *Staphylococcus aureus* (S. aureus), highlighting the advancements, methodologies, and outcomes of CRISPR-based interventions.

4.4.1. Mycobacterium tuberculosis: Enhancing Diagnostics and Therapeutics

Tuberculosis (TB), caused by Mtb, remains a leading cause of mortality worldwide. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has complicated treatment regimens [78].

CRISPR-Based Diagnostics:

Recent studies have leveraged CRISPR-Cas systems to develop rapid and sensitive diagnostic tools for TB. For instance, a CRISPR/Cas13a-based assay demonstrated high specificity and sensitivity in detecting Mtb DNA in clinical specimens, offering a promising alternative to traditional methods. Another approach combined recombinase-mediated isothermal amplification with CRISPR detection, achieving enhanced sensitivity for Mtb detection [79,80].

Therapeutic Applications:

Beyond diagnostics, CRISPR systems have been explored for therapeutic interventions. Harnessing the endogenous type III-A CRISPR/Cas10 system of Mtb, researchers achieved efficient gene editing and RNA interference, facilitating functional studies and potential therapeutic targets . Such advancements pave the way for novel treatments targeting essential genes in Mtb [78].

4.4.2. Escherichia coli: Combatting Antibiotic Resistance and Enhancing Detection

E. coli, a versatile bacterium, encompasses both commensal and pathogenic strains, with some exhibiting significant antibiotic resistance.

Preventing Antibiotic Resistance Acquisition:

In a study Lee et al [70] constructed a CRISPR-Cas9 system to protect probiotic E. coli strains from acquiring antibiotic resistance genes. By targeting specific resistance determinants, the system effectively prevented the horizontal transfer of resistance genes, maintaining the probiotic's susceptibility to antibiotics [70]

Enhanced Detection Methods:

CRISPR-Cas systems have also been employed to develop rapid and specific detection methods for pathogenic E. coli strains. A CRISPR-Cas12a-based assay demonstrated high sensitivity and specificity in identifying pathogenic E. coli isolates, facilitating timely diagnosis and intervention [80].

4.4.3. Staphylococcus aureus: Addressing Virulence and Resistance

S. aureus, particularly methicillin-resistant S. aureus (MRSA), poses significant challenges in clinical settings due to its virulence and resistance profiles [79].

Targeting Resistance Genes:

Researchers have utilized CRISPR-Cas systems to disrupt resistance genes in S. aureus. For example, CRISPR interference (CRISPRi) was employed to silence the nt5 gene, leading to reduced virulence and increased susceptibility to antibiotics.

Phage-Mediated CRISPR Delivery:

Innovative approaches have combined phage therapy with CRISPR systems. Phagemids delivering CRISPR components targeting essential genes in MRSA strains effectively reduced bacterial viability, showcasing a synergistic strategy to combat resistant infections [74,81].

5. Gene Editing in Vector-Borne Disease Control

Vector-borne diseases, notably malaria and dengue, continue to pose significant public health challenges globally, particularly in sub-Saharan Africa and parts of Asia [82]. Traditional control measures, including insecticide-treated nets and chemical spraying, have achieved varying degrees of success but face limitations due to insecticide resistance and behavioral adaptations of vectors. Advancements in gene editing technologies, especially CRISPR-Cas systems, have opened new avenues for controlling vector populations and interrupting disease transmission cycles [82]. This section explores the application of gene editing in vector control, focusing on the genetic modification of mosquito populations.

5.1. Genetic Modification of Mosquito Populations (e.g., *Anopheles* spp.)

Genetic modification of mosquitoes aims to reduce disease transmission by either suppressing vector populations or altering their capacity to transmit pathogens. *Anopheles* mosquitoes, the primary vectors of malaria, have been central to these genetic interventions [82].

5.1.1. Sterile Insect Technique (SIT) and Self-Limiting Genes

The Sterile Insect Technique involves releasing large numbers of sterile male mosquitoes into the wild. These males mate with wild females, resulting in no offspring and a subsequent decline in the mosquito population. Advancements have led to the development of genetically engineered mosquitoes carrying self-limiting genes. For instance, Oxitec has developed *Aedes aegypti* mosquitoes with a gene that causes female offspring to die before reaching maturity, effectively reducing the population over time. This approach has been piloted in various countries, including Brazil and the Cayman Islands, showing promising results in reducing mosquito populations [83,84].

5.1.2. Population Replacement Strategies

Instead of suppressing mosquito populations, population replacement strategies aim to introduce genetically modified mosquitoes that are incapable of transmitting pathogens. This involves inserting genes that render mosquitoes resistant to specific pathogens, such as the malaria parasite. By releasing these modified mosquitoes into the wild, the goal is for them to breed with wild populations, gradually replacing them with non-transmitting variants. Research has demonstrated the feasibility of this approach, with studies showing that genetically modified *Anopheles* mosquitoes can carry genes that block malaria transmission without affecting their survival or reproduction [85].

5.1.3. Field Trials and Implementation

Field trials are crucial for assessing the efficacy and safety of genetically modified mosquitoes. In 2024, Djibouti initiated a program to release genetically engineered non-biting *Anopheles stephensi* mosquitoes to combat urban malaria outbreaks. These mosquitoes are designed so that their female offspring do not survive, leading to a decline in the population. This initiative aims to reverse the resurgence of malaria cases in the region and serves as a model for other countries facing similar challenges [83].

5.2. Gene Drives for Malaria and Dengue Suppression

Gene drive technology represents a transformative approach in the fight against vector-borne diseases such as malaria and dengue. By biasing the inheritance of specific genes, gene drives can rapidly propagate desired traits through mosquito populations, either suppressing their numbers or rendering them incapable of transmitting pathogens. This section delves into the mechanisms, applications, and challenges associated with gene drives in controlling malaria and dengue [86].

5.2.1. Mechanisms and Applications of Gene Drives

Gene drives are genetic systems that increase the likelihood of a particular gene being inherited, surpassing the typical 50% inheritance rate observed in Mendelian genetics. Utilizing CRISPR-Cas9 technology, scientists can engineer gene drives that either suppress mosquito populations or modify them to be resistant to disease transmission [86].

In the context of malaria, gene drives have been designed to target genes essential for female fertility in *Anopheles* mosquitoes. By disrupting these genes, the reproductive capacity of female mosquitoes is compromised, leading to a decline in population over successive generations. Modeling studies have demonstrated that such gene drives could achieve up to 95% suppression of mosquito populations within four years, assuming optimal release strategies and minimal resistance development [87].

For dengue control, gene drives have been explored in *Aedes aegypti* mosquitoes. Strategies include introducing genes that confer resistance to the dengue virus or reduce the mosquitoes' lifespan to limit disease transmission. While laboratory studies have shown promise, field applications are still in nascent stages, necessitating further research to assess efficacy and safety in real-world settings [87].

5.2.2. Field Trials and Regulatory Considerations

Transitioning gene drive technologies from the laboratory to the field involves meticulous planning, regulatory approvals, and community engagement. Field trials are essential to evaluate the effectiveness, ecological impact, and potential risks associated with releasing gene drive mosquitoes into the environment.

In Africa, several initiatives have been undertaken to assess the feasibility of gene drives for malaria control. For instance, the Target Malaria consortium has conducted confined field trials to study the behavior and impact of gene drive mosquitoes. These trials are designed to simulate natural environments while ensuring containment and monitoring for unintended consequences [86].

Regulatory frameworks for gene drive technologies are still evolving. Given the potential for gene drives to spread across borders, international collaboration and harmonization of regulations are crucial. Ethical considerations, such as informed consent from affected communities and the potential ecological ramifications, must also be addressed to ensure responsible deployment [86].

5.2.3. Challenges and Future Directions

While gene drives offer a promising avenue for vector control, several challenges must be overcome to realize their full potential. One significant concern is the development of resistance within mosquito populations. Mutations that render the gene drive ineffective could emerge, necessitating the design of more robust and adaptable systems [86,88].

Ecological impacts are another area of concern. Suppressing or altering mosquito populations could have unforeseen effects on ecosystems, including the potential rise of other disease vectors filling the ecological niche left by targeted mosquitoes [88,89]. Comprehensive ecological assessments are essential to anticipate and mitigate such outcomes.

Looking ahead, advancements in gene editing technologies and a deeper understanding of mosquito genetics will enhance the precision and efficacy of gene drives. Collaborative efforts between scientists, policymakers, and communities will be pivotal in navigating the ethical, regulatory, and technical landscapes, ensuring that gene drives are harnessed responsibly in the global fight against vector-borne diseases.

5.3. Ecological and Ethical Considerations

The deployment of gene editing technologies, particularly gene drives, in vector-borne disease control introduces complex ecological and ethical dimensions. While the potential to eradicate diseases like malaria and dengue is compelling, the implications of releasing genetically modified organisms into the environment necessitate thorough examination. This section delves into the ecological risks, ethical debates, and the importance of community engagement associated with gene editing in mosquito populations.

5.3.1. Ecological Risks and Unintended Consequences

The ecological ramifications of releasing gene drive-modified mosquitoes are a subject of significant concern. Mosquitoes, despite their role as disease vectors, occupy integral positions in various ecosystems. Their larvae serve as food for aquatic organisms, while adult mosquitoes are prey for birds, bats, and other insects. Eliminating or altering mosquito populations could disrupt these food webs, leading to unforeseen ecological consequences [90]. Moreover, the specificity of gene drives raises questions about off-target effects. While designed to target particular species, there is a risk of gene flow to non-target species through hybridization, potentially affecting non-pest mosquito populations or other insects. Additionally, the long-term stability of gene drives in wild populations is uncertain. Mutations or evolutionary pressures could lead to resistance, diminishing the efficacy of the gene drive and possibly resulting in unintended genetic changes [90].

Environmental factors also play a role in the behavior of gene drive organisms. Variables such as climate, presence of predators, and availability of breeding sites can influence the spread and impact of genetically modified mosquitoes. Comprehensive ecological assessments and modeling are essential to predict and mitigate potential adverse outcomes before field releases are considered [85,91].

5.3.2. Ethical Debates Surrounding Gene Drive Technologies

The ethical considerations of employing gene drives in mosquito populations encompass a range of issues, from the moral status of mosquitoes to broader concerns about human intervention in nature. Some ethicists argue that deliberately causing the extinction of a species, even one as harmful as *Anopheles gambiae*, raises moral questions about human dominion over nature and the intrinsic value of all living organisms [92]. Conversely, proponents highlight the moral imperative to alleviate human suffering caused by vector-borne diseases. Malaria alone accounts for hundreds of thousands of deaths annually, predominantly in sub-Saharan Africa. From this perspective, the benefits of eradicating such diseases may outweigh the ethical concerns associated with gene drives. However, this utilitarian approach must be balanced against potential risks and the rights of communities affected by these interventions [93].

Another ethical dimension involves the allocation of resources. Investing in gene drive technologies may divert funds from other public health initiatives, such as improving healthcare infrastructure or education. Decisions about deploying gene drives should consider whether they represent the most effective and equitable use of limited resources, especially in regions where multiple health challenges coexist [93,94].

5.3.3. Community Engagement and Informed Consent

Engaging with communities is paramount when considering the release of gene drive-modified mosquitoes. Public perception, cultural beliefs, and local knowledge significantly influence the acceptance and success of such interventions. Historical instances, such as the Eliminate Dengue program, demonstrate that proactive community engagement can foster trust and support for vector control initiatives [95]. Informed consent is a critical ethical requirement, particularly when interventions directly impact individuals and communities. This includes transparency about the goals, methods, potential risks, and benefits of releasing genetically modified organisms. Obtaining consent becomes complex when interventions have transboundary effects or when individuals cannot opt out of exposure, necessitating broader public consultations and ethical deliberations [96].

Moreover, regulatory frameworks must ensure that community engagement is not merely procedural but genuinely incorporates local perspectives into decision-making processes. This involves establishing mechanisms for feedback, addressing concerns, and adapting strategies based on community input. Such participatory approaches are essential for the ethical deployment of gene drive technologies and for maintaining public trust in scientific interventions.

6. Future Perspectives and Emerging Trends

Table 6 Emerging Tools and Trends in Gene Editing for Infectious Disease Control

Technology	Description	Key Advantages	Future Potential Applications	Reference
Prime Editing	Cas9 nickase + reverse transcriptase guided by pegRNA	All base substitutions, no DSB	β-thalassemia, cystic fibrosis	[27, 35–36]
CRISPR-Cas13	RNA-targeting nuclease with collateral cleavage	Viral RNA degradation, diagnostics	SARS-CoV-2, Influenza	[56, 57]
SHERLOCK & DETECTR	CRISPR-based rapid nucleic acid detection platforms	Portable, highly specific	Field diagnostics for Ebola, Zika	[12, 17]
Synthetic Biology + CRISPR	Programmable immune responses	Custom-designed immune cells	HIV T-cell therapies, vaccines	[99–102]
AI-Optimized gRNA Design	Machine learning for guide optimization	High specificity, minimized off-targets	Personalized CRISPR interventions	[108–110]

As the field of CRISPR-Cas technology advances, future perspectives are increasingly focusing on how these systems can be integrated with other cutting-edge technologies, providing innovative solutions for global health challenges. The next

generation of CRISPR-based technologies and interdisciplinary integrations is poised to reshape the therapeutic landscape. Table 6 outlines cutting-edge innovations such as prime editing, RNA-targeting CRISPR systems, and AI-guided personalization, offering a glimpse into future directions for combating infectious diseases. This section also explores in detail some of the most promising future directions in the field, particularly in the context of synthetic biology, programmable immunity, AI-driven guide RNA design, integration with mRNA platforms, wearable diagnostics, and personalized interventions for infectious diseases.

6.1. Synthetic Biology and Programmable Immunity

The convergence of CRISPR technology with synthetic biology holds immense promise for creating programmable immunity, allowing for the precise control and modulation of immune responses. Synthetic biology refers to the design and construction of new biological parts, devices, and systems, which, when combined with CRISPR-based gene editing, could lead to the creation of programmable immune systems capable of responding to a wide range of pathogens [97,98]. This approach opens the possibility for next-generation vaccines, engineered immune cells, and even pathogen-resistant organisms.

6.1.1. Engineering Immunity Against Emerging Pathogens

One of the most exciting prospects of synthetic biology and CRISPR technology is the ability to engineer immune systems that are specifically tailored to combat emerging infectious diseases. Recent advances in synthetic biology have enabled the creation of custom-designed receptors and immune cells that can recognize and neutralize a variety of pathogens. CRISPR-based gene editing allows for the precise modification of immune cells, such as T-cells or B-cells, to recognize specific pathogens or their antigens [99,100]. For example, CRISPR has been used to engineer T-cells that can target HIV, potentially offering a novel approach to treating or even curing the disease. Additionally, the development of synthetic antibodies and immune modulators via CRISPR could enable personalized therapies against a broad range of infectious agents, from viruses to bacteria [101,102].

Programmable immunity could also be integrated into gene therapies, where CRISPR-edited immune cells are introduced into patients to fight chronic infections or even prevent future infections. For instance, in the case of malaria, synthetic biology could be used to engineer immune cells that target the Plasmodium parasite, potentially leading to long-lasting immunity. Such advancements would allow for more precise and individualized treatments, offering a highly specific therapeutic option for patients with various infectious diseases [103].

6.1.2. Synthetic Biology for Vaccine Development

Vaccines have been a cornerstone of global health, and their development has been significantly accelerated by synthetic biology. In combination with CRISPR-based systems, synthetic biology has opened new avenues for creating more effective and versatile vaccines. By using CRISPR to manipulate the genetic makeup of pathogens or viral vectors, researchers can design vaccines that elicit stronger and more targeted immune responses. This approach allows for the rapid design of vaccines against emerging infectious diseases, such as those caused by novel viruses or drug-resistant bacteria [104,105].

For example, the ability to synthetically construct viral vectors using CRISPR technology could lead to the development of more efficient vaccines. These vaccines could be designed to express specific antigens that prompt the immune system to mount a robust response. Additionally, synthetic biology could allow for the development of next-generation mRNA vaccines, which can be rapidly engineered to address a wide range of infectious agents. Such vaccines could be delivered more efficiently and tailored to individual patients, offering an adaptable and rapid response to new global health threats [106].

6.1.3. Ethical and Regulatory Considerations in Synthetic Biology

Despite the promising prospects, the integration of CRISPR-based synthetic biology into healthcare raises several ethical and regulatory concerns. One of the primary challenges is ensuring the safety and efficacy of genetically engineered immune cells or vaccines. As these technologies move into clinical use, it is essential to establish clear regulatory frameworks to evaluate the safety of synthetic biology-based therapies. Additionally, ethical questions surrounding gene editing, especially germline editing, and the potential for unintended consequences must be carefully considered. Public trust in these technologies will be vital, and transparent regulatory oversight will play a crucial role in their acceptance and implementation. Efforts to address these concerns through public dialogue and robust regulatory standards will be essential for realizing the potential of synthetic biology in programmable immunity [107].

6.2. AI-Driven Guide RNA Design for Pathogen Targeting

Artificial intelligence (AI) and machine learning (ML) are becoming increasingly integral to the advancement of CRISPR technology. In particular, AI-driven guide RNA (gRNA) design is one of the most promising areas for enhancing the precision and efficiency of CRISPR-based pathogen targeting. The CRISPR-Cas9 system relies on guide RNAs to direct the Cas9 protein to specific genomic locations, and the design of these guides is critical to the success of gene editing. AI can be utilized to predict and optimize gRNA sequences, improving their specificity and reducing off-target effects, thus enhancing the efficacy of pathogen-targeted CRISPR therapies [108,109].

6.2.1. Optimizing Guide RNA Design with AI

Traditionally, designing effective gRNAs for CRISPR-Cas9 applications has been a labor-intensive process, requiring careful consideration of factors such as sequence specificity and accessibility. However, AI algorithms, particularly deep learning models, have revolutionized this process by enabling the rapid analysis of large datasets to predict the most efficient gRNA sequences for any given target. These AI models take into account various factors, including DNA sequence context, chromatin accessibility, and potential off-target sites, making them invaluable tools for guiding gene editing efforts. With AI assistance, researchers can now design gRNAs that are more likely to achieve precise edits, even in complex pathogens such as viruses, bacteria, or parasites. These AI-optimized gRNAs have the potential to significantly improve the specificity and effectiveness of CRISPR-based gene therapies, particularly in the context of infectious diseases where accuracy is paramount [108].

6.2.2. AI Applications in Pathogen-Specific CRISPR Targets

AI-driven tools are also being used to identify pathogen-specific CRISPR targets, allowing for the development of highly targeted therapies against infectious diseases [108]. By analyzing the genomic sequences of pathogens, AI algorithms can pinpoint unique genetic markers that can be targeted with CRISPR-Cas9, ensuring that the intervention is both precise and effective. This approach is particularly useful for combating antibiotic-resistant bacteria, as AI can identify conserved genetic regions in resistant strains that are not present in non-resistant strains. This could lead to the development of CRISPR-based treatments that are effective against multi-drug-resistant pathogens, offering a powerful alternative to traditional antibiotics. Additionally, AI is being used to optimize CRISPR-based therapies for viral infections, where identifying specific viral sequences is crucial for effective gene editing interventions [110,111].

6.2.3. AI-Driven Personalization of Pathogen Treatment

The ability of AI to analyze vast amounts of genomic data also offers significant potential for personalizing CRISPR-based pathogen treatments. By integrating genomic information from individual patients with AI algorithms, healthcare providers could develop tailored therapies that target specific pathogens or strains present in a patient's body. This personalized approach could lead to more effective and targeted treatments, reducing the risk of off-target effects and improving patient outcomes. For example, in viral infections like HIV or hepatitis, personalized CRISPR therapies could be designed based on the specific viral strain present in the patient, increasing the chances of successful eradication and minimizing potential side effects. AI-driven personalization is an emerging frontier in infectious disease treatment, and its integration with CRISPR technology holds the potential to transform how we approach pathogen eradication [112,113].

6.3. Integration with mRNA Platforms and Wearable Diagnostics

The integration of CRISPR technologies with mRNA platforms represents an exciting frontier in biotechnology, particularly for infectious disease diagnosis, treatment, and personalized medicine. mRNA-based therapies, like those employed in COVID-19 vaccines, have demonstrated significant potential in rapidly producing vaccines and therapeutic agents. By combining CRISPR with mRNA technology, researchers can create highly adaptable, efficient systems capable of targeting specific pathogens or even genetic disorders with great precision. Additionally, wearable diagnostics, which are increasingly used to monitor health in real time, could benefit from the synergy of CRISPR and mRNA platforms, enabling continuous disease detection and therapeutic intervention [114].

6.3.1. mRNA as a Delivery Mechanism for CRISPR-Based Therapies

One of the most promising integrations of mRNA technology with CRISPR systems is the use of mRNA to deliver CRISPR components, including the Cas proteins and guide RNAs, directly into patient cells. This approach has the advantage of being less invasive and potentially more efficient than traditional gene delivery methods. mRNA vaccines, which have been successfully used for COVID-19, represent a platform that can be easily adapted for the delivery of CRISPR-based gene-editing tools. By encoding the Cas9 protein and gRNAs within mRNA sequences, scientists can deliver precise gene-editing instructions to cells in the body, enabling targeted modifications to the genome. This approach offers a rapid

and scalable means of treating genetic diseases, cancers, and infections, making it a key area of future development in both therapeutic and preventative medicine [114,115].

The combination of CRISPR with mRNA technology could also revolutionize vaccine development. For instance, researchers are exploring how mRNA could be used to instruct the body's cells to produce CRISPR proteins that target and edit the genomes of pathogens such as viruses or bacteria. This could provide a highly effective and dynamic method for combating infectious diseases, as the body could essentially "edit" the pathogens' genomes and neutralize them. mRNA vaccines that incorporate CRISPR components could also be quickly updated to address new viral variants, making them ideal candidates for dealing with rapidly evolving pathogens like the influenza virus or SARS-CoV-2 [116,117].

6.3.2. Wearable Diagnostics for Real-Time CRISPR-Based Monitoring

Wearable diagnostics, which monitor health indicators in real time, are another area where the integration of CRISPR and mRNA technologies holds great promise. These devices, which include smart watches and other wearable health monitors, could eventually track biomarkers associated with infectious diseases or genetic conditions. With the integration of CRISPR-based diagnostics, these wearables could detect the presence of specific pathogens or gene mutations by directly editing the genome or detecting genetic material related to the disease in real time. This could allow for personalized disease monitoring and the ability to initiate treatments (such as CRISPR-based therapies) at the earliest signs of illness, significantly improving outcomes [118].

For example, a CRISPR-powered wearable could track biomarkers of viral infections, such as the presence of viral RNA in a patient's body, and alert the individual to the need for further testing or immediate treatment. This would allow individuals to monitor their health continuously, providing an early warning system for potential infections or other health issues, ultimately leading to faster interventions and reduced transmission of infectious diseases. In addition, integrating CRISPR with wearable technology could enable the detection of cancer markers, genetic disorders, or immune system dysfunctions, offering continuous, personalized health monitoring [118,119].

6.3.3. Challenges and Future Directions in CRISPR-MRNA Integration

Despite the exciting possibilities, integrating CRISPR and mRNA technology with wearable diagnostics faces several challenges. For one, the delivery of CRISPR systems into the human body in a way that ensures efficient, specific, and safe gene editing remains a hurdle. While mRNA delivery platforms like lipid nanoparticles (LNPs) have shown promise, concerns about their long-term stability, immune responses, and potential off-target effects still persist. Furthermore, for CRISPR-based diagnostics to be effective, it will need to be highly sensitive and capable of detecting minute amounts of genetic material in real time without causing false positives or negatives [114]. Additionally, regulatory approval for CRISPR-based wearable diagnostics will require extensive validation to ensure safety and efficacy, especially given the novel nature of these combined technologies [109]. However, as research progresses and these challenges are addressed, the integration of CRISPR and mRNA platforms with wearable diagnostics has the potential to transform the way diseases are detected and treated.

6.4. Personalized Infectious Disease Interventions

The ability to personalize treatment based on an individual's genetic makeup is one of the most transformative aspects of modern medicine, and CRISPR technology is at the forefront of this revolution. Personalized infectious disease interventions refer to tailored treatments that consider not only the pathogen involved but also the patient's unique genetic, immune, and microbiome profiles. With CRISPR-based gene editing, it is now possible to target specific genes in both pathogens and patients to enhance treatment outcomes, reduce side effects, and address drug resistance [120]. As we move towards more precision-based medicine, CRISPR is playing a critical role in shaping the future of personalized therapies for infectious diseases.

6.4.1. Targeting Genetic Variability in Pathogens

Personalized infectious disease interventions often require the ability to address the genetic variability of pathogens, such as viruses, bacteria, and parasites, which can evolve rapidly to escape immune detection or become resistant to treatments. CRISPR-based systems provide a tool to directly target and edit the genome of these pathogens, allowing for personalized treatments that address the unique genetic makeup of the infectious agent. This approach is particularly useful for diseases caused by rapidly mutating pathogens, such as influenza, HIV, and tuberculosis. By utilizing CRISPR to edit the genes of these pathogens, it is possible to prevent or treat infections more effectively, even as the pathogens evolve. Personalized interventions can help address drug resistance, a significant challenge in treating infections like tuberculosis and HIV, by targeting the specific mutations that contribute to resistance [121].

Furthermore, by tailoring CRISPR-based therapies to individual patients, researchers can ensure that the treatment is highly specific and that it minimizes off-target effects. For instance, CRISPR can be used to target specific strains of bacteria that may cause chronic infections, while avoiding damage to the patient's beneficial microbiome. This level of precision is difficult to achieve with traditional antibiotic therapies, which can disrupt the microbiome and lead to side effects. Personalized CRISPR therapies, therefore, offer a more effective and targeted approach to infectious disease treatment [122].

6.4.2. Genetic and Immune Profiling for Tailored Treatments

Another key aspect of personalized infectious disease interventions is genetic and immune profiling. By understanding a patient's genetic makeup and immune response, it is possible to design CRISPR-based treatments that are optimized for their specific biology. For example, some individuals may have genetic variations that affect how their immune system responds to certain pathogens or how they metabolize drugs. CRISPR technology can be used to edit the patient's immune cells to enhance their response to infection or to correct genetic mutations that predispose them to certain diseases. For instance, CRISPR could be used to edit the genetic sequences of T-cells, making them more efficient at targeting and killing pathogens like HIV or malaria [123].

Similarly, profiling a patient's immune system could allow for more effective vaccines, designed to elicit stronger and more tailored immune responses. This personalized approach would take into account the individual's immune weaknesses, enhancing the body's ability to defend against infections. In addition, CRISPR can be used to target immune-related genes, providing a pathway to personalized immunotherapies that could be more effective for patients with specific genetic predispositions or immune deficiencies [124].

6.4.3. The Future of Personalized Medicine in Infectious Disease

Looking ahead, personalized infectious disease interventions will likely become a routine part of clinical practice, particularly with the continued advancements in CRISPR-based gene editing technologies. The ability to tailor treatments to an individual's specific genetic and immune profile will lead to more efficient, less toxic, and more effective treatments for a wide range of infectious diseases [125]. However, there are still challenges to overcome, including the need for better delivery systems for gene-editing tools, the ethical concerns associated with genetic modifications, and the regulatory hurdles associated with introducing personalized therapies into clinical practice. As research progresses and these challenges are addressed, CRISPR-based personalized treatments will become a cornerstone of precision medicine for infectious diseases, providing better outcomes for patients and helping to combat emerging and re-emerging infectious threats.

7. Conclusion

CRISPR-based gene editing technologies have shown immense potential in transforming the landscape of infectious disease control. With innovations in diagnostics, therapeutics, and vector population management, CRISPR systems have already demonstrated their ability to provide rapid and precise solutions to previously challenging health issues. The development of diagnostic tools like SHERLOCK and FELUDA has revolutionized pathogen detection, making it faster and more portable. Gene editing also holds promise for combating vector-borne diseases by modifying populations of mosquitoes to reduce transmission, while the use of CRISPR in therapeutics could provide tailored, more effective treatments for individuals.

However, despite these significant advancements, challenges remain. Efficient delivery systems, precise targeting, and minimizing off-target effects are critical areas that still need improvement. Ethical concerns surrounding the use of CRISPR, particularly in modifying organisms at the genetic level, continue to require careful regulation and oversight. Moving forward, it is essential for researchers to address these challenges while ensuring that gene editing technologies are accessible, safe, and beneficial for all populations. With continued research, collaboration, and ethical consideration, CRISPR holds great promise for the future of infectious disease control, paving the way for a new era in precision medicine and global health.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge the collaborative effort of all contributing scholars and colleagues who jointly authored and edited this review paper. This work was conducted entirely through the intellectual and academic

contributions of the authoring team, without external funding or assistance from any individual, institution, or organization.

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Zhang, Y., Zhang, Y., Chen, Z., Jia, Z., Yu, Y., Wang, J., & Liang, H. (2025). Global burden, subtype, risk factors and etiological analysis of enteric infections from 1990-2021: population based study. Frontiers in Cellular and Infection Microbiology, 15, 1527765.

[2] Burnham, C. A. D., Leeds, J., Nordmann, P., O'Grady, J., & Patel, J. (2017). Diagnosing antimicrobial resistance. Nature Reviews Microbiology, 15(11), 697-703.

[3] Cribbs, A. P., & Perera, S. M. (2017). Science and bioethics of CRISPR-Cas9 gene editing: an analysis towards separating facts and fiction. The Yale journal of biology and medicine, 90(4), 625.

[4] Wu, Y., & Zhang, H. (2023). Treatment of infectious diseases by in vivo gene editing. National Science Open, 2(5), 20220061.

[5] Hadipour, K., Asadishad, T., Mahya, L., Mohammadhassan, R., Rahbar, A., & Goudarziasl, F. (2023). A comparative review on genome editing approaches. Biointerface Res Appl Chem, 13(6), 567.

[6] Ghosh, S. K., & Chatterjee, T. (2024). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Associated Proteins (Cas)[CRISPR–Cas]: An Emerging Technique in Plant Disease Detection and Management. In Gene Editing in Plants: CRISPR-Cas and Its Applications (pp. 589-645). Singapore: Springer Nature Singapore.

[7] Asmamaw, M., & Zawdie, B. (2021). Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics: targets and therapy, 353-361.

[8] Li, T., Yang, Y., Qi, H., Cui, W., Zhang, L., Fu, X., ... & Yu, T. (2023). CRISPR/Cas9 therapeutics: progress and prospects. Signal transduction and targeted therapy, 8(1), 36.

[9] Zhang, H., Qin, C., An, C., Zheng, X., Wen, S., Chen, W., ... & Wu, Y. (2021). Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Molecular cancer, 20, 1-22.

[10] Li, G., Yang, X., Luo, X., Wu, Z., & Yang, H. (2023). Modulation of cell cycle increases CRISPR-mediated homology-directed DNA repair. Cell & Bioscience, 13(1), 215.

[11] Bandyopadhyay, A., Kancharla, N., Javalkote, V. S., Dasgupta, S., & Brutnell, T. P. (2020). CRISPR-Cas12a (Cpf1): a versatile tool in the plant genome editing tool box for agricultural advancement. Frontiers in plant science, 11, 584151.

[12] Nie, X., Wang, D., Pan, Y., Hua, Y., Lü, P., & Yang, Y. (2024). Discovery, classification and application of the CPISPR-Cas13 system. Technology and Health Care, 32(2), 525-544.

[13] Azeez, S. S., Hamad, R. S., Hamad, B. K., Shekha, M. S., & Bergsten, P. (2024). Advances in CRISPR-Cas technology and its applications: Revolutionising precision medicine. Frontiers in Genome Editing, 6, 1509924.

[14] Lai, M., Maori, E., Quaranta, P., Matteoli, G., Maggi, F., Sgarbanti, M., ... & Pistello, M. (2021). CRISPR/Cas9 ablation of integrated HIV-1 accumulates proviral DNA circles with reformed long terminal repeats. Journal of virology, 95(23), 10-1128.

[15] O'Hanlon Cohrt, K. (2023, November 1). Clinical trial update: Positive clinical data for first ever CRISPR therapy for HIV. CRISPR Medicine News. https://crisprmedicinenews.com/news/clinical-trial-update-positive-clinical-data-for-first-ever-crispr-therapy-for-hiv/?utm_source=chatgpt.com

[16] Sprayregen, M. (2021, October 29). A possible "functional cure" for HIV will soon begin human trials. Them. https://www.them.us/story/possible-functional-hiv-cure-will-begin-human-trials:contentReference[oaicite:3]{index=3}

[17] Tripathi, S., Khatri, P., Fatima, Z., Pandey, R. P., & Hameed, S. (2022). A landscape of CRISPR/Cas technique for emerging viral disease diagnostics and therapeutics: progress and prospects. Pathogens, 12(1), 56.

[18] Lin, H., Li, G., Peng, X., Deng, A., Ye, L., Shi, L., ... & He, J. (2021). The use of CRISPR/Cas9 as a tool to study human infectious viruses. Frontiers in Cellular and Infection Microbiology, 11, 590989.

[19] Azeez, S. S., Hamad, R. S., Hamad, B. K., Shekha, M. S., & Bergsten, P. (2024). Advances in CRISPR-Cas technology and its applications: Revolutionising precision medicine. Frontiers in Genome Editing, 6, 1509924.

[20] Porto, E. M., Komor, A. C., Slaymaker, I. M., & Yeo, G. W. (2020). Base editing: advances and therapeutic opportunities. Nature Reviews Drug Discovery, 19(12), 839-859.

[21] Chen, X., McAndrew, M. J., & Lapinaite, A. (2023). Unlocking the secrets of ABEs: the molecular mechanism behind their specificity. Biochemical Society Transactions, 51(4), 1635-1646.

[22] Newby, G. A., & Liu, D. R. (2021). In vivo somatic cell base editing and prime editing. Molecular Therapy, 29(11), 3107-3124.

[23] Hardouin, G., Antoniou, P., Martinucci, P., Felix, T., Manceau, S., Joseph, L., ... & Miccio, A. (2023). Adenine base editor–mediated correction of the common and severe IVS1-110 (G> A) β-thalassemia mutation. Blood, 141(10), 1169-1179.

[24] Zeng, J., Wu, Y., Ren, C., Bonanno, J., Shen, A. H., Shea, D., ... & Bauer, D. E. (2020). Therapeutic base editing of human hematopoietic stem cells. Nature Medicine, 26(4), 535-541.

[25] Zhou, J., Liu, Y., Wei, Y., Zheng, S., Gou, S., Chen, T., ... & Zou, Q. (2022). Eliminating predictable DNA off-target effects of cytosine base editor by using dual guiders including sgRNA and TALE. Molecular Therapy, 30(7), 2443-2451.

[26] Liao, J., Chen, S., Hsiao, S., Jiang, Y., Yang, Y., Zhang, Y., ... & Wu, Y. (2023). Therapeutic adenine base editing of human hematopoietic stem cells. Nature Communications, 14(1), 207.

[27] Anzalone, A. V., Gao, X. D., Podracky, C. J., Nelson, A. T., Koblan, L. W., Raguram, A., ... & Liu, D. R. (2022). Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. Nature biotechnology, 40(5), 731-740.

[28] Graff, G. D., & Sherkow, J. S. (2020). Models of technology transfer for genome-editing technologies. Annual Review of Genomics and Human Genetics, 21, 143–164. https://doi.org/10.1146/annurev-genom-121219-082856

[29] Huang, Z., & Liu, G. (2023). Current advancement in the application of prime editing. Frontiers in Bioengineering and Biotechnology, 11, 1039315.

[30] Murray, J. B., Harrison, P. T., & Scholefield, J. (2025). Prime editing: Therapeutic advances and mechanistic insights. Gene Therapy, 32(2), 83-92.

[31] Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., ... & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149-157.

[32] Yan, J., Oyler-Castrillo, P., Ravisankar, P., Ward, C. C., Levesque, S., Jing, Y., ... & Adamson, B. (2024). Improving prime editing with an endogenous small RNA-binding protein. Nature, 628(8008), 639-647.

[33] Wikipedia contributors. (n.d.). Prime editing. Wikipedia. Retrieved May 12, 2025, from https://en.wikipedia.org/wiki/Prime_editing

[34] Ochoa-Sanchez, A., Perez-Sanchez, G., Torres-Ledesma, A. M., Valdez, J. P. R., Rinaldi, G., Moguel, B. B., & Molina-Aguilar, C. (2021). Prime Editing, a novel genome-editing tool that may surpass conventional CRISPR-Cas9. Re: GEN Open, 1(1), 75-82.

[35] Everette, K. A., Newby, G. A., Levine, R. M., Mayberry, K., Jang, Y., Mayuranathan, T., ... & Liu, D. R. (2023). Ex vivo prime editing of patient haematopoietic stem cells rescues sickle-cell disease phenotypes after engraftment in mice. Nature Biomedical Engineering, 7(5), 616-628.

[36] Sousa, A. A., Hemez, C., Lei, L., Traore, S., Kulhankova, K., Newby, G. A., ... & Liu, D. R. (2025). Systematic optimization of prime editing for the efficient functional correction of CFTR F508del in human airway epithelial cells. Nature biomedical engineering, 9(1), 7-21.

[37] Petrova, I. O., & Smirnikhina, S. A. (2023). The development, optimization and future of prime editing. International Journal of Molecular Sciences, 24(23), 17045.

[38] Wolff, J. H., & Mikkelsen, J. G. (2023). Prime editing in hematopoietic stem cells—From ex vivo to in vivo CRISPR-based treatment of blood disorders. Frontiers in Genome Editing, 5, 1148650.

[39] Davis, J. R., Banskota, S., Levy, J. M., Newby, G. A., Wang, X., Anzalone, A. V., ... & Liu, D. R. (2024). Efficient prime editing in mouse brain, liver and heart with dual AAVs. Nature biotechnology, 42(2), 253-264.

[40] Glass, Z., Lee, M., Li, Y., & Xu, Q. (2018). Engineering the delivery system for CRISPR-based genome editing. Trends in biotechnology, 36(2), 173-185.

[41] Stewart, M. P., Sharei, A., Ding, X., Sahay, G., Langer, R., & Jensen, K. F. (2016). In vitro and ex vivo strategies for intracellular delivery. Nature, 538(7624), 183-192.

[42] Li, L., & Mandal, P. K. (2024). Recent advancements in gene therapy for sickle cell disease and β-thalassemia. Frontiers in Hematology, 3, 1468952.

[43] Dever, D. P., Bak, R. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. E., ... & Porteus, M. H. (2016). CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature, 539(7629), 384-389.

[44] Li, Y., Glass, Z., Huang, M., Chen, Z. Y., & Xu, Q. (2020). Ex vivo cell-based CRISPR/Cas9 genome editing for therapeutic applications. Biomaterials, 234, 119711.

[45] Yang, X., Bui, T. A., Mei, H., Aksoy, Y. A., Deng, F., Hutvagner, G., & Deng, W. (2024). Exploring the Potential and Challenges of CRISPR Delivery and Therapeutics for Genetic Disease Treatment. Advanced Functional Materials, 34(38), 2402630.

[46] Wei, T., Cheng, Q., Farbiak, L., Anderson, D. G., Langer, R., & Siegwart, D. J. (2020). Delivery of tissue-targeted scalpels: opportunities and challenges for in vivo CRISPR/Cas-based genome editing. ACS nano, 14(8), 9243-9262.

[47] Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. (2018). Delivering CRISPR: a review of the challenges and approaches. Drug delivery, 25(1), 1234-1257.

[48] Finn, J. D., Smith, A. R., Patel, M. C., Shaw, L., Youniss, M. R., van Heteren, J., ... & Morrissey, D. V. (2018). A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell reports, 22(9), 2227-2235.

[49] Wang, D., Zhang, F., & Gao, G. (2020). CRISPR-based therapeutic genome editing: strategies and in vivo delivery by AAV vectors. Cell, 181(1), 136-150.

[50] Wang, H., Lu, H., Lei, Y. S., Gong, C. Y., Chen, Z., Luan, Y. Q., ... & Zhang, W. F. (2020). Development of a self-restricting CRISPR-Cas9 system to reduce off-target effects. Molecular Therapy Methods & Clinical Development, 18, 390-401.

[51] Chehelgerdi, M., Chehelgerdi, M., Khorramian-Ghahfarokhi, M., Shafieizadeh, M., Mahmoudi, E., Eskandari, F., ... & Mokhtari-Farsani, A. (2024). Comprehensive review of CRISPR-based gene editing: mechanisms, challenges, and applications in cancer therapy. Molecular cancer, 23(1), 9.

[52] Bayoumi, M., & Munir, M. (2021). Potential use of CRISPR/cas13 machinery in understanding virus–host interaction. Frontiers in Microbiology, 12, 743580.

[53] Escalona-Noguero, C., López-Valls, M., & Sot, B. (2021). CRISPR/Cas technology as a promising weapon to combat viral infections. BioEssays, 43(4), 2000315.

[54] Sadowski, I., & Hashemi, F. B. (2019). Strategies to eradicate HIV from infected patients: elimination of latent provirus reservoirs. Cellular and Molecular Life Sciences, 76, 3583-3600.

[55] Martinez, M. G., Boyd, A., Combe, E., Testoni, B., & Zoulim, F. (2021). Covalently closed circular DNA: the ultimate therapeutic target for curing HBV infections. Journal of hepatology, 75(3), 706-717.

[56] Zhang, Y., & Li, M. (2021). Genome editing technologies as cellular defense against viral pathogens. Frontiers in Cell and Developmental Biology, 9, 716344.

[57] Levy, S. (2020, March 18). Could Crispr be humanity's next virus killer? WIRED. https://www.wired.com/story/could-crispr-be-the-next-virus-killer/?utm_

[58] Wilbie, D., Walther, J., & Mastrobattista, E. (2019). Delivery aspects of CRISPR/Cas for in vivo genome editing. Accounts of chemical research, 52(6), 1555-1564.

[59] Rosanwo, T. O., & Bauer, D. E. (2021). Editing outside the body: ex vivo gene-modification for β-hemoglobinopathy cellular therapy. Molecular Therapy, 29(11), 3163-3178.

[60] Kulkarni, C. R., Prajnashree, A., & Nyamagoud, S. B. (2025). CRISPR Technology in Antiviral Therapy: Advancements, Applications, and Implications. DY Patil Journal of Health Sciences, 13(1), 25-31.

[61] Mullin, E. (2022, October 5). A bold effort to cure HIV—using CRISPR. WIRED. https://www.wired.com/story/a-bold-effort-to-cure-hiv-using-crispr/

[62] Fischer, W., Giorgi, E. E., Chakraborty, S., Nguyen, K., Bhattacharya, T., Theiler, J., ... & Korber, B. (2021). HIV-1 and SARS-CoV-2: Patterns in the evolution of two pandemic pathogens. Cell host & microbe, 29(7), 1093-1110.

[63] Pacia, D. M., Brown, B. L., Minssen, T., & Darrow, J. J. (2024). CRISPR-phage antibacterials to address the antibiotic resistance crisis: scientific, economic, and regulatory considerations. Journal of Law and the Biosciences, 11(1), lsad030.

[64] Gencay, Y. E., Jasinskytė, D., Robert, C., Semsey, S., Martínez, V., Petersen, A. Ø., ... & Sommer, M. O. A. (2024). Engineered phage with antibacterial CRISPR–Cas selectively reduce E. coli burden in mice. Nature Biotechnology, 42(2), 265-274.

[65] Khambhati, K., Bhattacharjee, G., Gohil, N., Dhanoa, G. K., Sagona, A. P., Mani, I., ... & Singh, V. (2023). Phage engineering and phage-assisted CRISPR-Cas delivery to combat multidrug-resistant pathogens. Bioengineering & Translational Medicine, 8(2), e10381.

[66] Stanley, S. Y., & Maxwell, K. L. (2018). Phage-encoded anti-CRISPR defenses. Annual review of genetics, 52(1), 445-464.

[67] Ekwebelem, O. C., Aleke, J., Ofielu, E., & Nnorom-Dike, O. (2021). CRISPR-cas9 system: A revolutionary tool in the fight against antimicrobial resistance: retracted. Infectious Microbes & Diseases, 3(2), 51-56.

[68] Mayorga-Ramos, A., Zúñiga-Miranda, J., Carrera-Pacheco, S. E., Barba-Ostria, C., & Guamán, L. P. (2023). CRISPR-Cas-based antimicrobials: design, challenges, and bacterial mechanisms of resistance. ACS infectious diseases, 9(7), 1283-1302.

[69] Allemailem, K. S. (2024). Recent advances in understanding the molecular mechanisms of multidrug resistance and novel approaches of CRISPR/Cas9-based genome-editing to combat this health emergency. International Journal of Nanomedicine, 1125-1143.

[70] Lee, D., Muir, P., Lundberg, S., Lundholm, A., Sandegren, L., & Koskiniemi, S. (2025). A CRISPR-Cas9 system protecting E. coli against acquisition of antibiotic resistance genes. Scientific Reports, 15(1), 1545.

[71] Molteni, M. (2019, January 16). Antibiotics are failing us. CRISPR is our glimmer of hope. WIRED. Retrieved from https://www.wired.com/story/antibiotics-are-failing-us-crispr-is-our-glimmer-of-hope/

[72] Bikard, D., & Barrangou, R. (2017). Using CRISPR-Cas systems as antimicrobials. Current opinion in Microbiology, 37, 155-160.

[73] Palacios Araya, D., Palmer, K. L., & Duerkop, B. A. (2021). CRISPR-based antimicrobials to obstruct antibiotic-resistant and pathogenic bacteria. PLoS Pathogens, 17(7), e1009672.

[74] Mullin, E. (2024, September 10). Crispr-enhanced viruses are being deployed against UTIs. WIRED. Retrieved from https://www.wired.com/story/crispr-enhanced-viruses-are-being-deployed-against-utis/

[75] Nath, A., Bhattacharjee, R., Nandi, A., Sinha, A., Kar, S., Manoharan, N., ... & Suar, M. (2022). Phage delivered CRISPR-Cas system to combat multidrug-resistant pathogens in gut microbiome. Biomedicine & Pharmacotherapy, 151, 113122.

[76] Abavisani, M., Faraji, N., Faraji, S., Ebadpour, N., Kesharwani, P., & Sahebkar, A. (2024). A comprehensive review on utilizing CRISPR/Cas system for microbiome modification. Biochemical Engineering Journal, 109443.

[77] Ahmed, M. M., Kayode, H. H., Okesanya, O. J., Ukoaka, B. M., Eshun, G., Mourid, M. R., ... & Lucero-Prisno III, D. E. (2024). CRISPR-Cas Systems in the Fight Against Antimicrobial Resistance: Current Status, Potentials, and Future Directions. Infection and Drug Resistance, 5229-5245.

[78] Rahman, K., Jamal, M., Chen, X., Zhou, W., Yang, B., Zou, Y., ... & Cao, G. (2022). Reprogramming Mycobacterium tuberculosis CRISPR system for gene editing and genome-wide RNA interference screening. Genomics, Proteomics & Bioinformatics, 20(6), 1180-1196.

[79] Ren, W., Zhou, Y., Li, H., Shang, Y., Zhang, X., Yuan, J., ... & Pang, Y. (2023). Development and clinical evaluation of a CRISPR/Cas13a-based diagnostic test to detect Mycobacterium tuberculosis in clinical specimens. Frontiers in microbiology, 14, 1117085.

[80] Zhang, X., He, X., Zhang, Y., Chen, L., Pan, Z., Huang, Y., & Li, H. (2023). A new method for the detection of Mycobacterium tuberculosis based on the CRISPR/Cas system. BMC Infectious Diseases, 23(1), 680.

[81] Roberts, R. (2024, November 18). Tackling antibiotic-resistant Staphylococcus aureus with phagemids and Cas13a. CRISPR Medicine News. Retrieved from https://crisprmedicinenews.com/news/tackling-antibiotic-resistant-staphylococcus-aureus-with-phagemids-and-cas13a/?

[82] Oliver, S., & Raman, J. (2024, September 23). Genetically modified mosquitoes could strengthen war on malaria. Alliance for Science. Retrieved from https://allianceforscience.org/blog/2024/09/genetically-modified-mosquitoes-could-strengthen-war-on-malaria/

[83] Financial Times. (2024, May 23). Djibouti looks to genetically engineered mosquitoes to quell urban malaria wave. https://www.ft.com/content/b8a1fbef-be76-4ccf-a7b8-68dbcd8fd377

[84] Centers for Disease Control and Prevention. (2024, April 20). Genetically modified mosquitoes. Centers for Disease Control and Prevention. https://www.cdc.gov/mosquitoes/mosquito-control/genetically-modified-mosquitoes.html

[85] Moreno, R. D., Valera, L., Borgoño, C., Castilla, J. C., & Riveros, J. L. (2024). Gene drives, mosquitoes, and ecosystems: an interdisciplinary approach to emerging ethical concerns. Frontiers in Environmental Science, 11, 1254219.

[86] Simoni, A. (2021, September 6). Gene drive mosquitoes successfully control mosquito populations in large cages. Target Malaria. Retrieved from https://targetmalaria.org/latest/blog/gene-drive-mosquitoes-successfully-control-mosquito-populations-in-large-cages/

[87] North, A. R., Burt, A., & Godfray, H. C. J. (2020). Modelling the suppression of a malaria vector using a CRISPR-Cas9 gene drive to reduce female fertility. BMC biology, 18, 1-14.

[88] Connolly, J. B., Burt, A., Christophides, G., Diabate, A., Habtewold, T., Hancock, P. A., ... & Randazzo, F. (2024). Considerations for first field trials of low-threshold gene drive for malaria vector control. Malaria journal, 23(1), 156.

[89] Campbell-Lendrum, D., Molyneux, D., Amerasinghe, F., Davies, C., Fletcher, E., Schofield, C., ... & Epstein, P. (2005). Ecosystems and vector-borne disease control. Ecosystems and human well-being: policy responses, 3, 353-372.

[90] STOP GENE DRIVES. (n.d.). Ecological risks. STOP GENE DRIVES. Retrieved May 12, 2025, from https://www.stop-genedrives.eu/en/ecological-risks/?utm_source=chatgpt.com

[91] National Academies of Sciences, Engineering, and Medicine. (2016). Chapter 6: Assessing risks of gene-drive modified organisms. In Gene drives on the horizon: Advancing science, navigating uncertainty, and aligning research with public values (pp. 112–130). The National Academies Press. https://doi.org/10.17226/23405

[92] Sarkar, S. (2016, April 19). The ethics of gene drives against Aedes aegypti. Healio. https://www.healio.com/news/infectious-disease/20160419/the-ethics-of-gene-drives-against-aedes-aegypti

[93] Wise, I. J., & Borry, P. (2022). An ethical overview of the CRISPR-based elimination of Anopheles gambiae to combat malaria. Journal of Bioethical Inquiry, 19(3), 371-380.

[94] Emerson, C., James, S., Littler, K., & Randazzo, F. (2017). Principles for gene drive research. Science, 358(6367), 1135-1136.

[95] Teem, J. L., Ambali, A., Glover, B., Ouedraogo, J., Makinde, D., & Roberts, A. (2019). Problem formulation for gene drive mosquitoes designed to reduce malaria transmission in Africa: results from four regional consultations 2016–2018. Malaria journal, 18, 1-13.

[96] Kolopack, P. A., & Lavery, J. V. (2017). Informed consent in field trials of gene-drive mosquitoes. Gates open research, 1, 14.

[97] Jeong, S. H., Lee, H. J., & Lee, S. J. (2023). Recent advances in CRISPR-Cas technologies for synthetic biology. Journal of Microbiology, 61(1), 13-36.

[98] Katz, L., Chen, Y. Y., Gonzalez, R., Peterson, T. C., Zhao, H., & Baltz, R. H. (2018). Synthetic biology advances and applications in the biotechnology industry: a perspective. Journal of Industrial Microbiology and Biotechnology, 45(7), 449-461.

[99] Jin, K., Huang, Y., Che, H., & Wu, Y. (2025). Engineered Bacteria for Disease Diagnosis and Treatment Using Synthetic Biology. Microbial Biotechnology, 18(1), e70080.

[100] Kim, S., Hupperetz, C., Lim, S., & Kim, C. H. (2021). Genome editing of immune cells using CRISPR/Cas9. BMB reports, 54(1), 59.

[101] Herrera-Carrillo, E., Gao, Z., & Berkhout, B. (2020). CRISPR therapy towards an HIV cure. Briefings in functional genomics, 19(3), 201-208.

[102] Binnie, A., Fernandes, E., Almeida-Lousada, H., De Mello, R. A., & Castelo-Branco, P. (2021). CRISPR-based strategies in infectious disease diagnosis and therapy. Infection, 49, 377-385.

[103] Goswami, D., Minkah, N. K., & Kappe, S. H. (2019). Designer parasites: genetically engineered Plasmodium as vaccines to prevent malaria infection. The Journal of Immunology, 202(1), 20-28.

[104] Tan, X., Letendre, J. H., Collins, J. J., & Wong, W. W. (2021). Synthetic biology in the clinic: engineering vaccines, diagnostics, and therapeutics. Cell, 184(4), 881-898.

[105] Shirley, J. L., De Jong, Y. P., Terhorst, C., & Herzog, R. W. (2020). Immune responses to viral gene therapy vectors. Molecular Therapy, 28(3), 709-722.

[106] Travieso, T., Li, J., Mahesh, S., Mello, J. D. F. R. E., & Blasi, M. (2022). The use of viral vectors in vaccine development. npj Vaccines, 7(1), 75.

[107] Gyngell, C., Douglas, T., & Savulescu, J. (2017). The ethics of germline gene editing. Journal of Applied Philosophy, 34(4), 498-513.

[108] Varshney, N., Patidar, N., Dixit, S. K., & Jain, R. (2025). AI-Powere d CRISPR: Revolutionizing Precision Medicine and Genomi c Therapeutics. Am J Biomed Sci Res, 26(2).

[109] Hassan, Y. M., Mohamed, A. S., Hassan, Y. M., & El-Sayed, W. M. (2025). Recent developments and future directions in point-of-care next-generation CRISPR-based rapid diagnosis. Clinical and Experimental Medicine, 25(1), 1-15.

[110] Boretti, A. (2024). The transformative potential of AI-driven CRISPR-Cas9 genome editing to enhance CAR T-cell therapy. Computers in Biology and Medicine, 182, 109137.

[111] Olatunji, A. O., Olaboye, J. A., Maha, C. C., Kolawole, T. O., & Abdul, S. (2024). Next-Generation strategies to combat antimicrobial resistance: Integrating genomics, CRISPR, and novel therapeutics for effective treatment. Engineering Science & Technology Journal, 5(7), 2284-2303.

[112] Segun, A. F. (2024). Advances in personalized medical therapeutics: Leveraging genomics for targeted treatments. International Journal of Research Publication and Reviews, 5(10), 2921-2933.

[113] Quazi, S. (2022). Artificial intelligence and machine learning in precision and genomic medicine. Medical Oncology, 39(8), 120.

[114] Parhiz, H., Atochina-Vasserman, E. N., & Weissman, D. (2024). mRNA-based therapeutics: looking beyond COVID-19 vaccines. The Lancet, 403(10432), 1192-1204.

[115] Wilbie, D., Walther, J., & Mastrobattista, E. (2019). Delivery aspects of CRISPR/Cas for in vivo genome editing. Accounts of chemical research, 52(6), 1555-1564.

[116] Alavi, M., & Rai, M. (2021). Antisense RNA, the modified CRISPR-Cas9, and metal/metal oxide nanoparticles to inactivate pathogenic bacteria. Cellular, Molecular and Biomedical Reports, 1(2), 52-59.

[117] Kennedy, E. M., & Cullen, B. R. (2015). Bacterial CRISPR/Cas DNA endonucleases: A revolutionary technology that could dramatically impact viral research and treatment. Virology, 479, 213-220.

[118] Sharma, A., Badea, M., Tiwari, S., & Marty, J. L. (2021). Wearable biosensors: an alternative and practical approach in healthcare and disease monitoring. Molecules, 26(3), 748.

[119] Haghayegh, F., Norouziazad, A., Haghani, E., Feygin, A. A., Rahimi, R. H., Ghavamabadi, H. A., ... & Salahandish, R. (2024). Revolutionary Point-of-Care Wearable Diagnostics for Early Disease Detection and Biomarker Discovery through Intelligent Technologies. Advanced Science, 11(36), 2400595.

[120] Allemailem, K. S. (2024). Recent advances in understanding the molecular mechanisms of multidrug resistance and novel approaches of CRISPR/Cas9-based genome-editing to combat this health emergency. International Journal of Nanomedicine, 1125-1143.

[121] Kwok, A. J., Mentzer, A., & Knight, J. C. (2021). Host genetics and infectious disease: new tools, insights and translational opportunities. Nature Reviews Genetics, 22(3), 137-153.

[122] Ramachandran, G., & Bikard, D. (2019). Editing the microbiome the CRISPR way. Philosophical Transactions of the Royal Society B, 374(1772), 20180103.

[123] Kotowski, M., & Sharma, S. (2020). CRISPR-based editing techniques for genetic manipulation of primary T cells. Methods and protocols, 3(4), 79.

[124] Feng, X., Li, Z., Liu, Y., Chen, D., & Zhou, Z. (2024). CRISPR/Cas9 technology for advancements in cancer immunotherapy: from uncovering regulatory mechanisms to therapeutic applications. Experimental Hematology & Oncology, 13(1), 102.

[125] Esfahani, K., Elkrief, A., Calabrese, C., Lapointe, R., Hudson, M., Routy, B., ... & Calabrese, L. (2020). Moving towards personalized treatments of immune-related adverse events. Nature reviews Clinical oncology, 17(8), 504-515.