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CRISPR-Cas9 GENE EDITING: MECHANISMS, CLINICAL APPLICATIONS, AND FUTURE PERSPECTIVES

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ABSTRACT

The system of gene editing known as the CRISPR-Cas9 system has revolutionized the field of gene editing and provides a platform that allows for the modification of genetic sequences with precision and efficiency, having implications for both the basic research of science and for treatments. Its mechanism, which is derived from bacterial adaptive immunity, consists of a guide RNA that leads to specific epigenetic DNA sites a nuclease named Cas, to target it for cleavage; this permits gene correction, inactivation, or insertion. The clinical potential of this technology is enormous with applications ranging from hereditary diseases, oncology, and infectious diseases. Of note too, the approvals from the FDA for therapies targeted at the treatment of sickle cell anemia and beta thalassemia speaks volumes to its transformative effect on what was previously in-treatable conditions. In oncology, the technologies are increasing immunotherapies by engineering T-cells and interfering with immune checkpoints, but also working on viral genomes (e.g. HIV, HBV) and destroying antibiotic-resistance genes in bacteria. However, there are still major challenges such as off-target effects, in vivo delivery efficiency and possible immunogenicity of Cas proteins. Future advancements are dedicated to refine the use of the CRP systems using advanced variants such as base and prime editors, and to extend the toolkit by Cas12 and Cas13 for RNA/DNA targeting, as well as devising innovative delivery systems for overcoming the biological barriers. Ethical, regulatory, and societal considerations are paramount in the continued promotion and progressive growth of this technology.

KEYWORDS

CRISPR-Cas9, Oncology, Infectious Diseases, Gene Editing Technologies, Personalized Therapy

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Introduction

Development of accurate gene-editing tools has been a longstanding goal in the life sciences, as the ability to manipulate genetic sequences with extreme efficiency and specificity has attracted a great deal of interest (Zheng et al., 2024). Over a period of almost seventy years since the elucidation of the structure of the DNA double-helix, various techniques have been developed to progressively enhance methods for manipulating the genome, ranging from crude homologous recombination to sophisticated instruments such as zinc finger nucleases and transcription activator-like effector nucleases. (Zheng et al., 2024). The development of the CRISPR - Cas system and in particular of the Cas9 protein has revolutionized genetic engineering by providing a highly versatile platform for site-controlled modifications such as insertions, deletions and replacements of DNA (Zheng et al., 2024).

Overview of Gene Editing Technologies

This is a technology based on a programmable RNA-guided nuclease to create induced double-strand breaks in the genome in order to achieve specific correction of pathogenic mutations or regulation of gene function (Park et al., 2025). Such capability has tremendous fulfillment for both fundamental research, which allows for intricate dissection of genetic pathways, as well as for therapeutic applications, which might represent the first possibility to address genetic diseases in their root cause (Deneault, 2024). The ease of engineering and multiplexing provided by the use of the CRISPR/Cas9 system has made it the central tool to develop models of disease and investigate gene therapy approaches (Eid & Mahfouz, 2016). Its impressive efficiency and programmability have pushed the use of the so-called CRISPR technologies to the forefront of the genomic revolution, allowing the opening of new perspectives for understanding and treating complex biological processes (Pacesa et al., 2024). urther exploration into various types of CRISPR - Cas 12 and 13 enzymes have significantly expanded the range of gene editing capabilities to allow for more complex genetic modifications and therapeutic interventions(Deneault, 2024).

Historical Context of CRISPR-Cas Systems

Early attempts of modifying the genomic DNA were fundamental but suffer from low efficiency and lack of specificity as opposed to today's precision that can be achieved using advanced nucleases (Abhijith & Thangavel, 2025). The discovery of the groundbreaking technology of the so-called CRISPR - Cas systems, which were first identified as adaptive immune systems in bacteria and archaea against viral infections and plasmids, was a key paradigm shift, as this represents a naturally evolved solution for specific targeting of DNA (Janik et al., 2020; Zhang, 2019). This bacterial defense mechanism, co-opted for genome engineering, uses a guide RNA to direct the activity of a Cas nuclease to specific DNA sequences in order to use targeted DNA cuts that can be repaired by non homo-ologous end joining or homology-directed repair (McGrail et al., 2022). The ability to induce site specific double stranded breaks has made highly specific genetic changes, including gene knockouts, specific insertions and correction of disease causing mutations (Natanzi et al., 2025). The inherent simplicity and programmability of the CRISPR/Cas9 system, which is based on an RNA-guided mechanism instead of labor-intensive protein engineering for sequence binding domains, is also a huge differentiator from previous gene-editing tools such as zinc finger nucleases and TALEN (Xu et al., 2024).

Mechanism of CRISPR-Cas Systems

The CRISPR-Cas system, a very sophisticated adaptive immune machinery that is conserved in prokaryotic organisms, is a formidable defense against invading mobile genetic elements, including viruses and plasmids (Wang et al., 2022; Xu & Li, 2020) This system has revealed the exquisite immunological strategies used by bacteria and archaea and has been ingeniously adapted to an invaluable tool for precise genome editing which has had a profound impact on the advancement of biotechnology and therapeutic interventions (Moon et al., 2019; Wang et al., 2022). The basic operational architecture of the CRISPR-Cas

systems involves three main steps: adaptation, crRNA biogenesis and interference (Makarova et al., 2025; Nussenzweig & Marraffini, 2020). Adaptation is essential to establish immune memory in the CRISPR-Cas system, in this stage the short genetic fragments (called protospacer) from invading pathogens are incorporated into the host genome's CRISPR locus, a locus comprised of direct repeats interspersed with unique spacer sequences that represent historical infections (Lee & Sashital, 2022; Navarro et al., 2025). Integration is mediated mostly by the Cas1 and Cas2 proteins, which form a complex that cuts out and inserts these foreign fragments of DNA (Kumari et al., 2021). This precise incorporation mechanism allows the host to recognize and neutralize later encounters with identical invaders, which is often supplemented with primed adaptation which efficiently acquires new spacers from previously encountered pathogens, which boosts the immune response (Lee & Sashital, 2022; Nussenzweig & Marraffini, 2020). After adaptation the integrated spacer sequences are transcribed into a long precursor of the CRISPR RNA (pre-cRNA) that is further processed during crRNA biogenesis to generate mature, short length, CRISPR RNAs (crRNAs). In Type II systems, for example, a trans-activating crRNA (tracrRNA) binds to pre-crRNA to form a double stranded RNA which is subsequently cleaved by host RNAse 3 in combination with Cas9 to yield mature crRNAs that are crucial for the guiding of Cas effector proteins to their specific nucleic-acid targets (Xu & Li, 2020). The interference stage is the effector phase; it is during this stage that host actively neutralizes the invading genetic elements. Mature crRNA binds to Cas effector proteins to form a ribonucleoprotein complex that searches the cellular environment for nucleic acid sequences that are complementary to the crRNA spacer (Nussenzweig & Marraffini, 2020). A key determinant of target recognition in many CRISPR-Cas systems is the protospacer adjacent motif (PAM), which is a short nucleotide sequence located adjacent to the protospacer in the invading DNA and serves as a key targeting element in the Cas reaction as well as a mechanism of self versus non-self discrimination against autoimmunity (Collias & Beisel, 2021; Javaid et al., 2022). Upon successful recognition and binding of the crRNA-Cas complex to a complementary target sequence and its associated PAM, the Cas effector protein initiates cleavage of the foreign nucleic acid. The exact cleavage mechanism varies by system; in the well-characterized Class 2 Type II CRISPR-Cas9 system, the Cas9 protein, guided by the single guide RNA (sgRNA), induces double-stranded breaks in the target DNA, typically three base pairs upstream of the PAM (Mengstie & Wondimu, 2021). These double - stranded breaks are then repaired through cellular repair processes, often leading to gene inactivation through non - homologous end joining or precision gene editing when a homologous gene repair template is available for homology directed gene repair (Zhou & Yao, 2023). CRISPR-Cas systems have tremendous-diversity and are broadly categorized into two major classes (Class 1 and Class 2), then divided into many types and subtypes with two distinct molecular architectures and target strategies (Koonin et al., 2023; Makarova et al., 2025). Class 1 systems use multi-proteins effector complexes for interference (Liu & Doudna, 2020). For example, Type I systems use a Cascade complex for target recognition followed by the helicase-nuclease Cas3 for the degradation of foreign DNA (Yoshimi et al., 2022). Type III systems are unprecedented in their ability to target both RNA and single-stranded DNA (ssDNA); target RNA cleavage is often coupled to RNA-activated indiscriminate ssDNA cleavage (Lin et al., 2020; Stella & Marraffini, 2023). These systems also include the synthesis of cyclic oligoadenylate (cOA) secondary messengers by Cas10 subunit leading to the activation of ancillary effector proteins to enhance the immune response (Jungfer et al., 2025; Paraan et al., 2023); however, it is noteworthy that Type III CRISPR-Cas effectors are protein assisted ribozymes during RNA cleavage (Taylor et al., 2023). In contrast, Class 2 systems are defined by one large effector protein that arbitrates the target recognition as well as cleavage (Koonin et al., 2023). This structural simplicity has made Class 2 systems, especially Type II, Type V and Type VI, very attractive for genome editing (Moon et al., 2019). Cas12 nucleases, for example, initiate DNA cleavage by forming a R-loops, and cause consecutive staggered cleavage of the target DNA (Cofsky et al., 2020; Losito et al., 2021; Naqvi et al., 2022). Cas 13 subtypes, on the other hand, specifically recognize and cleave RNA, which initiates a non-selective RNase activity that cleaves the targeted RNA (cis - cleavage), and other RNA molecules (trans - cleavage) (Kimchi et al., 2023; Kuo et al., 2024). Ongoing research is uncovering the structural and functional intricacies of these diverse systems and provide insights into their evolutionary origins and expand the toolbox available for genome engineering(Koonin et al., 2023; Wang et al., 2022). A thorough knowledge of the dynamic mechanisms of different Cas proteins, including their capacity to recognize PAM sequences and cleave nucleic acids, is of utmost importance for basic scientific discovery as well as for the development of novel biotechnological applications (Gleditzsch et al., 2018; Yoshimi et al., 2022). In conclusion, the complex molecular mechanisms of CRISPR-Cas systems, including the fine-tuned adaptation, RNA guide biogenesis, and interference, highlight their role as a prokaryote immune system and their transformative impact on genetic engineering and biomedical research (Pandey et al., 2025; Xu et al., 2025).

Continued investigation of these mechanisms promises further developments in our ability to effectively manipulate genomes and fight diseases.

Clinical applications of CRISPR-Cas9

The genome-editing system known as the Cas9/CpR system has revolutionised the world of clinical medicine, from being a tool used for laboratory research into a tool that has been used to treat a host of human diseases with precision and targeted changes to the human genome (Deneault, 2024; Zhou & Yao, 2023). This very powerful technology is being explored for its potential to prevent or treat many inherited and acquired illnesses, such as various cancers, hemolytic disorders, immunodeficiencies, cardiovascular diseases, visual impairments, neurodegenerative disorders, and various X-linked disorders (Khoshandam et al., 2023). Compared to previous genome-editing methods, the main features of the technique, namely simplicity, efficiency and excellent specificity, have allowed the introduction into clinical trials of this technology (Sharma et al., 2020). The therapeutic potential of the technology, referred to as 'CRISPR-Cas9', spans a wide range of genetic diseases, including malignancies, allergies, immunological diseases, Duchenne muscular dystrophy, cardiovascular diseases, neurological diseases, hepatic diseases, cystic fibrosis, hematological diseases, ocular diseases, and viral infections (Abdelnour et al., 2023; Khan et al., 2018; Sharma et al., 2020). Beyond direct therapeutic uses, the development of the technology (CRISPR/-Cas9) has a major contribution to disease modelling and can allow a better understanding of the mechanism underlying different infectious and genetic diseases, which can then be used to develop more effective treatments (Khan et al., 2018). A landmark in this regard was the approval by the US Food and Drug Administration (FDA) of Casgevy, a gene-editing therapy using the CRISP-Cas9, in late 2023 for the treatment of both sickle-cell anaemia and transfusion-dependent beta-thalassaemia, thereby indicating the immense impact that this gene-editing therapy will have on diseases previously refractory to treatment (Bharti & Mudge, 2025; Leonard & Tisdale, 2024; Parums, 2024; Rahmat et al., 2024). Casgevy specifically uses the technique of CRISPR-Cas9 editing at the erythroid-specific enhancer of the BCL11A gene to boost fetal hemoglobin production, which shows the system's ability to achieve therapeutic outcomes with the precision targeting of specific genetic elements (Leonard & Tisdale, 2024). This approval, together with Lyfgenia for sickle-cell disease, is a dual breakthrough for gene therapies, as Casgevy is the first gene therapy using the newly developed gene editing tool, called CRISPR, to be approved by the FDA (Leonard & Tisdale, 2024; Rahmat et al., 2024). Beyond these approved therapies there are clinical trials broadly exploring the use of CRISPR-based therapeutics in a range of other conditions, including specific trials into HIV and a range of cancers (Bharti & Mudge, 2025; Mengstie & Wondimu, 2021). For example, the use of CRISPR/Cas9 in clinical trial for non-small cell lung cancer in which T-cells are edited ex vitro to target PD-1 and other trials for renal cell carcinoma where CAR-T cell therapy is combined with the disruption of CD70, TRAC and B2M to induce disease stability and in some patients, sustained remission (Allemailem et al., 2024). Further trials are ongoing for advanced stage refractory cancers, including the deletion of TRAC and TRBC genes from T-lymphocytes to promote anti-cancer immunity and the introduction of transgenes such as NYESO-1 to improve recognition of cancers (Allemailem et al., 2024). Furthermore, research is being conducted into the usage of the gene-editing tool, called CRISPR/Cas9, to correct specific mutations in genes of patients in gene therapy, giving hope to diseases that could not be cured by traditional methods, such as Leber congenital amaurosis (Morshedzadeh et al., 2023). These therapeutic strategies include both ex vivo gene editing, in which cells are harvested, genetically modified in vitro, expanded and then re-introduced into the patient, and in vivo gene editing, in which the effectors of a gene-editing strategy are delivered directly into the patient's body and into targeted tissues or organs (Hirakawa et al., 2020; Palacios et al., 2024). The ex vivo editing strategy has shown promising preliminary data in a variety of clinical trials, especially in conditions such as sickle cell disease and transfusion dependent beta-thalassemia with the pioneering phase 3 trials of CXT001 using a CRISPR-Cas9 based ex vivo gene editing strategy (Hirakawa et al., 2020; Huang et al., 2022). However, the therapeutic usefulness of ex vivo approaches is limited by the availability of some tissues and organs for cell harvesting and reintroduction (Palacios et al., 2024). On the other hand, in vivo systems based on the use of lentiviral vectors (CRP) that are used in vivo have the potential to revolutionize the treatment of a much wider range of diseases by specifically addressing the specific mutations directly in the body; for example, if we focus on hepatocytes, we could treat metabolic diseases (Huang et al., 2022; Palacios et al., 2024). Despite incredible advancements, the large-scale clinical implementation of CRISPR/Cas9 is faced with numerous challenges, such as immunogenicity concerns, the need for better delivery systems, potential off-target effects and various ethical considerations (Hirakawa et al., 2020; Mengstie & Wondimu, 2021; Morshedzadeh et al., 2023). Safely and

efficiently delivering the CRISPR/Cas9 system to target cells is a major hurdle in in vivo gene editing because the human immune system may mount a response to bacterial-derived Cas9 (Brandes et al., 2021; Li et al., 2023; Wang, 2020). Current strategies involve improving delivery modalities, including viral vehicles and non-viral delivery such as nanoparticles, to be more efficient and safer when it comes to therapeutic gene editing and improve its clinical translatability (Deneault, 2024; Foley et al., 2022; Khlidj, 2023). Addressing off-target editing, inefficient delivery and counterproductive immune responses continues to be the focus of ongoing research (Hirakawa et al., 2020).

Clinical Applications of CRISPR-Cas9 in Oncology

The CRISPR-Cas9 genome editing system has fundamentally changed the view of oncology, offering a powerful, precise, and versatile tool for revlutionary cancer research, improved diagnostic capabilities, and the development of innovative therapeutic strategies (Deng, 2024; Liu et al., 2021; Park et al., 2025). This technology allows for unprecedented ability of manipulation for genetic sequences that offers greater insight into the molecular basis of cancer progression and enabling the generation of complex genetic models for drug discovery and drug development (Bhat et al., 2022; Katti et al., 2022; Sharma & Giri, 2024; Wang et al., 2022; Zhao et al., 2021). One of the cornerstones of the clinical application of the gene editing technology CRISPR-Cas9 in the field of oncology is its ability to boost cancer immunotherapy, in particular through the engineering of immune cells. For example, chimeric antigen receptor T-cell (CAR-T) therapy has shown remarkable success in treating hematologic malignancies, thanks to the capabilities of modifying the T-cells with the aid of the methods enabled by the gene-editing technique of clustered regularly interspaced short palindromic nucleotide (CRP-Cas9) (Li et al., 2024; Qiu et al., 2022; Zhang et al., 2024). Precise gene disruption of genes like PD-1, CTLA-4 or LAG-3 enables the use of the Cas9 enzyme of the immune checkpoint inhibitor (CRP-Cas9) that is capable of breaking through the immune checkpoints that inhibit the function of T-cells, thus enabling a stronger antitumor immune response (Allemailem et al., 2023; Bishnoi, 2023; Feng et al., 2024; Ou et al., 2021). This approach not only enhances CAR-T cell expansion and persistence but also creates immune reaction sensitivity in tumour cells, which then increases the effectiveness of existing immunotherapies (Feng et al., 2024; Liu et al., 2023).

Beyond immunotherapy, the direct application of the CRISPR-Cas9 system in therapeutic interventions to correct cancer driving genetic mutations and modulate tumor microenvironment is increasingly being studied. It provides the opportunity to inactivate oncogenes (e.g. BCR -ABL1 in chronic myelogenous leukemia or JAK2 in myeloproliferative disorders) or restore tumor suppressor gene function, directly inhibiting cancer progression (Ikhtiar et al., 2025; Li et al., 2024; Park et al., 2025). Such precise manipulation of genes is instrumental in the overcoming of therapeutic resistance (a significant obstacle in oncology) through manipulation of genes that drive resistance to chemotherapy, radiotherapy and immunotherapy to improve overall treatment effectiveness (Li et al., 2024; Park et al., 2025). The innovative potential of the technique goes to the preparation of components for adoptive cell transfer immunotherapy; several such strategies have already reached the FDA approval process, ushering in a new era in cancer management (Allemailem et al., 2023).

Numerous clinical trials are currently underway, investigating the safety and efficacy of therapies based on the use of the technology described above in the treatment of a wide range of malignancies. These trials include investigations of relapsed or refractory B- cell malignancies using T cells genetically engineered with the Cas9 enzyme (CRP-SP); studies of non-small cell lung cancer and renal cell carcinoma metastases using T cells that have been ex vivo modified with an antibody against the enzyme PD-1 (Ahumada-Ayala et al., 2023; Allemailem et al., 2024; Bhattacharjee et al., 2022; Chen et al., 2022; Jachowski et al., 2023; Kanbar et al., 2024). Other studies include metastatic colorectal cancer, glioblastoma, sarcoma, multiple myeloma, prostate cancer, acute myeloid leukemia and various solid tumors, including targets such as specific oncogenes (e.g. KrAS, ALK, NRAS, IDH1, epidermal growth factor receptor (EGFR)), tumor suppressor genes (e.g. TP53, PTEN) and genes involved in the tumor microenvironment or drug resistance (e.g. MUC1, CCR4, HIF-1a, TGF-beta receptor II, DNMT1, LAP) (Chehelgerdi et al., 2024). What is important, the first-in-human phase I trial using PD-1 T cells editing with the same technology (CRISPR-Cas9) in patients with advanced stage of non-small cell lung cancer (NSC) demonstrated feasibility and general safety with a low frequency of off-target mutations (Allemailem et al., 2024; Jachowski et al., 2023). The ability to edit T cells to express chimeric antigen receptors or T - cell receptors against tumor antigens such as NY - ES0 - 1 or CD19, as seen in trials for metastatic melanoma, non-Hodgkin's lymphoma and acute lymphoblastic leukemia, is a major step forward in personalized cancer therapy (Becerra et al., 2022; Chehelgerdi et al., 2024).

Despite the transformative potential of the technology, there are several technical and biological challenges associated with the use of CRISPR-Cas9 in oncology that must be overcome for widespread clinical use of the technology. Key issues include off-target effects, where unwanted genomic changes occur, and complex issues of efficient and safe in vivo delivery of the components of the CRISPR system to the target cells (Allemailem et al., 2024; Chehelgerdi et al., 2024; Hirakawa et al., 2020; Karimi et al., 2025; Li et al., 2024; Mengstie & Wondimu, 2021; Zhang et al., 2024). Immunogenicity - i.e. the host immune response against Cas9 proteins derived from bacteria - is a major obstacle that may affect the therapeutic efficacy and lead to adverse reactions (Chen et al., 2023; Ebrahimi et al., 2023; Kanbar et al., 2024). Ongoing research aims to address these limitations through the development of high fidelity Cas9 variants, optimized guide RNA designs, and innovative delivery systems such as lipid nanoparticles and engineered viral vectors designed for enhanced specificity and reduced immunogenicity (Foley et al., 2022; Kanbar et al., 2024; Rostami et al., 2024; Sinclair et al., 2023). While moving from bench to bedside is still ongoing, the incredible progress and innovations of the past and the future of this technology make CRISPR-Cas9 a revolutionary force set to redefine cancer diagnosis, treatment and fundamental disease understanding, ushering in the era of precision oncology (Chaudhary et al., 2021; Chehelgerdi et al., 2024; Deng, 2024).

Clinical Applications of CRISPR-Cas9 in Infectious Diseases

The genome editing system of the Cas9/CRP sequence has been deeply changing the clinical methods for infectious diseases with unprecedented precision and versatility in a field that is often plagued by the evolution of pathogens, as well as the growing drug resistance that has emerged (Abdelnour et al., 2023; Bharti & Mudge, 2025; Liu et al., 2021). This technology is now being repurposed to directly target and neutralise invading pathogens or to genetically engineer host cells to provide proper resistance to infection (Escalona-Noguero et al., 2021; Hawsawi et al., 2022; Lin et al., 2021). Its simplicity, efficiency, and high specificity allow researchers to make specific changes to DNA and RNA, making the use of the technique (CRISPR-Cas) a powerful weapon against a wide range of infectious agents, such as viruses, bacteria, and fungi, and for the development of advanced diagnostics (Al-Ouqaili et al., 2025; Dubey & Mostafavi, 2023).

In the case of viral infections, however, the capabilities of the CRISPR-Cas systems are dual action, allowing both direct attack of viral genomes and also modification of cellular factors in the host cell that are essential for viral replication. For example, CSI/CRP9 is leading the charge to fight human immunodeficiency virus, or HIV, a threat that is affecting people worldwide, creating latent reservoirs in infected cells (Hawsawi et al., 2022). Clinical trials are currently exploring the potential of using CRISPR/Cas9 to reduce these reservoirs by targeting the CCR5 gene in the host cell, a co-receptor needed for HIV infection of CD4+ T-lymphocytes (Bellizzi et al., 2019; Bharti & Mudge, 2025; Mengstie & Wondimu, 2021). This approach imitates a natural CCR5- mutations (CCR5-Delta 32) that gives resistance to HIV- this highlights the ability of the technology called CRISPR to reproduce protective genetic variations. Moreover, the integrase system (CRISPR/Cas9) can be employed to specifically target and inactivate integrated HIV-1 proviruses, and this provides hope for a functional cure in patients taking antiretroviral therapy (Bellizzi et al., 2019). Hepatitis B virus is also a similarly important target because of its persistence, mainly occurring as covalently closed circular DNA (cccDNA) in infected hepatocytes (Galy et al., 2023). Preclinical research shows that the technologies of the gene-editing approach of genetic modification (CRISPR/Cas) can be used to accurately remove or disrupt this cccDNA to restrict viral replication and potentially overcome chronic HBV infection (Galy et al., 2023). In addition, RNA-targeting CRISPR systems such as LshCas13a have been shown to be effective in the inhibition of human papillomavirus (HPV) associated cervical cancer by cleavage of specific HPV mRNA transcripts in vivo (Ding et al., 2021). Beyond these, the potential of using CRISPR/Cas9 has been explored for emerging viral diseases including the current pandemic, and Cas13a has been successfully used to inhibit Dengue virus replication by targeting its NS3 gene (Ding et al., 2021). The versatility of unique Cas proteins - DNA - targeting Cas9/Cas12 and RNA - targeting Cas13 - offers a complete arsenal against a wide variety of viral pathogens and their unique replication strategies (Escalona-Noguero et al., 2021; Wu & Zhang, 2023).

The challenge of bacterial infections and the growing crisis of antimicrobial resistance are other important areas where the transformative potential of the CRISPR-Cas technology is being explored. CRISPR mediated antimicrobials have high specificity and can specifically kill the pathogenic bacteria while leaving the commensal microbiota unaffected, which is a major advantage over the conventional antibiotics (Benz et al., 2025; Dubey & Mostafavi, 2023). Engineered systems of the biotechnological tools known as Cas9-enzyme (CRP) could kill bacteria directly or, importantly, reverse bacterial resistance to antibiotics and

effectively "resensitize" them to existing drugs (Al-Fadhli & Jamal, 2024; Wu et al., 2021). This is accomplished by the targeted and precise deletion of antibiotic resistance genes, either on plasmids (such as MCR-1 colistin resistance genes from Escherichia coli and Klebsiella pneumoniae) or embedded in the bacterial chromosome (such as tetM and ermB resistance genes from Enterococcus faecalis) (Ahmed et al., 2024). Specific constructs of the protein Cas9, as well as Cas13a-based nucleocapsids, have shown the ability to target virulence genes, thereby disarming pathogens and preventing disease, while other is able to kill resistant bacteria in a sequence-specific manner by recognizing and cleaving antimicrobial resistance gene transcripts (Butiuc-Keul et al., 2021; Tagarro et al., 2024). Such highly specific strategies are of great value in the control of multidrug-resistant pathogens, such as the notorious ESKAPE group, which is a major cause of hospital-acquired infections and is currently almost untreatable with existing antibiotics (Qian et al., 2023).

Such highly specific strategies have a great potential in the control of multidrug-resistant pathogens, particularly members of the ESKAPE group, which is one of the main causes of nosocomial infections and is currently nearly invincible to existing antibiotics. A major concern is the possible off-target effects, where undesirable genetic changes can lead to negative developments (Guo et al., 2023; Karimi et al., 2025). The safe and efficacious delivery of the components of the CRISPR system to desired tissues or cells is another obstacle that must be overcome, and this requires the development of innovative vectors that can address the problem of degradation, non-specific distribution and immunogenicity related to bacterial-derived Cas proteins (Guo et al., 2024; Liu et al., 2025; Rostami et al., 2024; Sinclair et al., 2023). These technical and biological limitations highlight the need for ongoing research and development to ensure the safety and efficacy of therapeutics based on the use of the gene-editing technology.

Looking to the future, the perspectives for the technology of the future with regards to the use of CRISPR-Cas are characterised by relentless innovation. This trajectory entails the development of next generation of the protein engineering of the Cas system, such as base and prime editors, which provide better control of genetic modifications, and the investigation of various Cas enzymes including Cas12 and Cas13 to enrich the DNA and RNA targeting ability (Deneault, 2024; Villiger et al., 2024; Zheng et al., 2024). Advances in delivery system and specifically delivery using non-viral nanoparticles, and advanced viral vectors for clinical use are pivotal in the clinical applicability (Madigan et al., 2023; Rostami et al., 2024). The combination of the potential of creating more specific and accurate designs using artificial intelligence and the potential of using them in diagnostics and epigenome editing promises to speed up its use in diagnostics and epigenome editing (Hassan et al., 2025). Eighteenth Ideas and Implications Turning these all promising means of collaboration and innovation into real public health solutions ultimately rests on the ethics, regulation, and social responsibility of using this powerful technology innovatively, to achieve that elusive perfect balance between revolutionary potential and tremendous societal responsibility (Anliker et al., 2022; Biswas, 2025; Li et al., 2024).

Conclusions

The development of the CRISPR-Cas9 system has had a tremendous impact on biomedicine, as it has transformed from a bacterial defense mechanism to a versatile gene-editing tool. Its precision allows for groundbreaking therapeutic applications in fields ranging from genetic disorders to oncology and infectious diseases (e.g. FDA approved therapies to treat Sickle Cell disease). Despite the game-changing potential, there are still challenges in reducing the off-target effects, increasing the delivery efficiency and dealing with immunogenicity. Continued innovation in next generation of the technology for the development and use of radiation- and toxicity-free of the late generation are the tools and strategies of the next generation, and are coupled to careful navigation of the ethical and regulatory landscapes. This is to ensure that the revolutionary power of CRISPR-Cas9 is used responsibly to guarantee equitable access and that the greatest beneficial impact is achieved in the global health.

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