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Review

CRISPR-Cas9 Applications in Gene Therapy: Advances, Challenges, and Future Perspectives

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Abstract

CRISPR-Cas9 has rapidly emerged as a gold standard for accurate genome editing, which boasts remarkable potential for human therapies. This brief review article summarizes the most up-to-date developments in CRISPR-Cas9 gene therapy with a focus on single-gene disorders such as sickle cell disease, β-thalassemia, and Leber congenital amaurosis. We explain how Cas9 generates a double-stranded break in DNA and how subsequent repair leads to gene correction. We describe base and prime editing methods that raise precision and drastically reduce off-target effects. Still, one of the most significant challenges in the therapeutic context is the delivery of the editing machinery to the desired cells. There are many approaches under investigation, such as the use of adeno-associated viral vectors, lipid nanoparticles or electroporation, which are being evaluated for their efficacy, safety, and their ability to home in on the right tissue. Immunogenicity, undesired mutations, and long-term genetic stability remain major concerns. Besides the technical concerns, we also go over the ethical and legal issues, such as the germline gene editing, equitable access, and informed consent, which emphasize the whole world perspective on responsible use. Eventually, the therapeutic scope of CRISPR-Cas9 may be expanded by joining forces with RNA-targeting technologies, epigenetic modulators, and AI-based design software. Despite the hurdles, CRISPR-Cas9 is expected to revolutionize the field of precision medicine, thereby providing extraordinary possibilities for safe and efficacious genome-based medical interventions.

Keywords

CRISPR, CRISPR-Cas9, Gene therapy, Precision medicine, Base and prime editing, Genome editing

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1. Introduction

1.1 Overview of CRISPR-Cas9 Technology

Gene therapy is a treatment method that modifies genes by replacing, correcting, or interrupting defective regions, providing new hope to patients with genetic illnesses who previously had few treatment options. Early gene therapy depended heavily on viral vectors to deliver therapeutic genes, with one of the first clinical trials using *ex vivo* retroviral delivery of a neomycin-resistance marker into tumor-infiltrating leukocytes from patients with metastatic melanoma. While this strategy was not immediately therapeutic, it did mark a significant milestone in the clinical application of gene therapy [1].

The emergence of the CRISPR-Cas9 system has transformed gene therapy by providing a precise and versatile tool for gene editing. CRISPR-Cas9 was first discovered in bacteria and archaea in 1987 as a virus and plasmid transfer defensive mechanism [2]. It allows for targeted genome alteration to fix defective genes. Its applications go beyond medicine, including building disease-resistant crops in plant biotechnology and developing animal models with precise genetic changes for research [3].

Despite its potential, gene therapy poses problems such as efficiently and safely delivering genetic material to target cells, reducing off-target effects, and negotiating ethical and regulatory constraints—particularly for germline editing. Overcoming these difficulties is important to the widespread adoption of gene treatments [4].

1.2 Significance of Gene Therapy

The introduction of CRISPR-Cas9 into gene therapy has changed the field by allowing for direct, precise genome editing. Unlike earlier techniques that focused on delivering functional copies of genes, CRISPR-Cas9 allows for permanent change of the patient's own DNA, perhaps leading to long-term treatments rather than symptom management. This method has important implications for treating rare genetic illnesses such as sickle cell anemia, cystic fibrosis, and muscular dystrophy. CRISPR-Cas9-based medicines also show promise for targeted cancer treatment and other genetic illnesses, giving patients and researchers hope.

1.3 Purpose and Scope of Review

This mini-review highlights the evolution of the CRISPR-Cas9 applications as gene therapy and presents the current state of key clinical activities, preclinical results, and technical progression in advancing this technology of gene-editing. The review will also address areas of concern and drawbacks of the CRISPR-based therapeutics and offer insight into future innovation and a possible broadening of CRISPR-Cas9 use in therapeutic management.

2. Methodology

A comprehensive search of electronic databases such as PubMed, Web of Science, and Scopus was used to identify relevant literature for this review. Keywords and search phrases included "gene therapy", "CRISPR-Cas9", "genome editing", "genetic disorders", and "therapeutic applications", which were coupled with Boolean operators (AND, OR) to refine results. Articles published from 2015 to 2025 were evaluated. The reference lists of selected papers were further reviewed to locate more relevant studies. Inclusion criteria emphasized original research, clinical trials, and high-quality reviews on CRISPR-Cas9 applications in gene therapy and associated difficulties, while studies with insufficient data or unrelated themes were rejected.

3. Current Progress in CRISPR-Cas9 Gene Therapy

3.1 Clinical Applications of CRISPR-Cas9 in Genetic Diseases

CRISPR-Cas9 is under development in several clinical trials to treat inherited diseases. Blood disorders such as sickle cell anemia and beta thalassemia is one of the most promising fields [5]. In these diseases, malfunctions occur in the hemoglobin gene and produce distorted red blood cells, resulting in acute symptoms. Additionally, CRISPR-Cas9 has been employed to edit hematopoietic stem cells (HSCs), making those cells healthy to replace in the patient. In 2020, a striking study showed that CRISPR-based treatment may provide a potential cure to sickle cell anemia, and an enzyme system called CRISPR-based treatment can be directly applicable to patients who have a genetic mutation [6]. *Ex vivo* CRISPR-Cas9 gene editing allows for precise genome change under controlled settings, as shown in Figure 1.

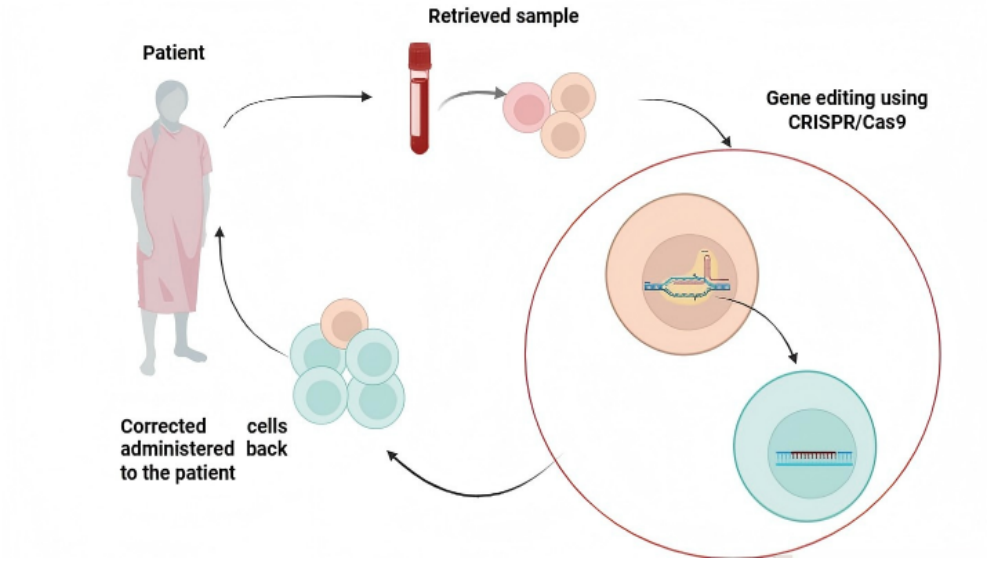

Figure 1. *Ex Vivo* CRISPR-Cas9 gene editing workflow.

Figure 1 depicts the process of *ex vivo* gene therapy with CRISPR-Cas9. First, cells are extracted from a patient and collected as a specimen. In the laboratory, CRISPR-Cas9 technology is used to edit and correct defective genes in these cells. The repaired cells are then cultured and processed. Finally, the patient receives healthy, modified cells to treat the hereditary condition.

The second direction of improvement is the treatment of muscular dystrophy, which is a set of genetic disorders resulting in the degradation of muscles. Recently, it has been noted that CRISPR-Cas9 may be applied to change the flawed gene of dystrophin (the genesis of Duchenne muscular dystrophy) in the animal model. These make CRISPR-Cas9 a promising tool with the potential to reverse or mitigate the effects of patients with genetic muscle diseases [7].

Exagamglogene autotemcel (CTX001), a CRISPR-based gene-editing treatment that targets the erythroid-specific enhancer of BCL11A, was evaluated in crucial clinical trials by Vertex Pharmaceuticals. In this *ex vivo* method, autologous $CD34^+$ haematopoietic stem and progenitor cells were electroporated with CRISPR-Cas9 editing components. These cells were then reinfused into patients with sickle cell disease (SCD; NCT03655678) or transfusion-dependent β-thalassemia (TDT; NCT03745287). Six months following therapy, the trial showed over 86% on-target editing in bone marrow $CD34^+$ cells in SCD patients, along with maintained foetal haemoglobin (HbF) levels ≥40% during follow-up. Clinically, for more than 12 months in a row, 97% of treated patients did not experience any vaso-occlusive periods. Rather than CRISPR-mediated genome editing, busulfan-based myeloablative conditioning was the primary cause of reported adverse effects. Nine months after starting therapy, one patient died, and the cause was coronavirus disease 2019 (COVID-19). A significant advancement in the clinical translation of CRISPR-based treatments for SCD was made in 2023 when the United States Food and Drug Administration approved this haematopoietic stem cell gene-editing medication targeting the BCL11A erythroid enhancer under the name CASGEVY [8].

3.2 Technological Advances and Improvements in CRISPR-Cas9

Although the original CRISPR-Cas9 technology had demonstrated an innovative way to perform gene-editing, issues associated with specificity and off-target editing are common. In counter response, there exist several advancements to the safety and the effectiveness of gene editing. Other technologies, such as base editing and prime editing, have taken shape as a more specific alternative to conventional CRISPR-Cas9. Delivery remains a major obstacle in the clinical translation of CRISPR-Cas9-based therapeutics, with numerous techniques established to transport Cas9 and guide RNAs into target cells. Figure 2 illustrates the various delivery modalities, such as viral vectors, lipid nanoparticles, and physical methods, and highlights their modes of cellular entry [9].

3.3 Base Editing

Base editing is a modified CRISPR technology that allows for accurate single-nucleotide conversions without causing double-strand DNA breakage. It uses a catalytically inhibited Cas9 coupled to a nucleotide deaminase to enable for direct chemical alteration of target bases within a specific editing window. Cytosine base editors facilitate C-G to T-A transitions, while adenine base editors convert A-T to G-C [9]. This method lowers insertion-deletion formation compared to standard CRISPR-Cas9, making it ideal for repairing harmful point mutations that cause monogenic illnesses like SCD and β-thalassemia. However, base editing is restricted to specific transition mutations and sequence contexts. Bystander modifications within the editing window, as well as the constrained target scope, remain significant constraints [10].

3.4 Prime Editing

A more flexible method of genome engineering, prime editing may introduce exact base substitutions, insertions, and deletions without the need for donor DNA templates or double-strand breaks. It makes use of a primary editing guide RNA that codes for the intended change and a Cas9 nickase attached to a reverse transcriptase enzyme [11].

Prime editing can treat a wider range of pathogenic variations than base editing, which is restricted to transition mutations. In comparison to traditional CRISPR-Cas9 systems, it exhibits lower rates of indel generation and decreased off-target activity. However, the comparatively large size of the editing equipment, inconsistent editing efficiency, and delivery complexity now limit its clinical translation [12].

3.5 Comparative Perspective

Conventional CRISPR-Cas9 is still very effective for general gene disruption or insertion techniques, but it has a larger chance of indel generation and off-target consequences due to double-strand break repair processes. Base editing improves precision for single-nucleotide repairs, while prime editing provides the most flexibility for complicated genomic changes. Current research focuses on increasing efficiency, reducing inadvertent edits, and enhancing delivery systems for clinical scalability [13]. Therefore, Table 1 summarizes the key differences among CRISPR-Cas9, base editing, and prime editing.

Table 1. Comparison of CRISPR gene editing technologies.

Technology	Precision	Off-Target Effects	Applications	Limitations	Ref
CRISPR-Cas9	Moderate	Higher	General gene editing, gene therapy	Off-target mutations, scalability issues	[14]
Base Editing	High	Low	Correcting point mutations	Limited target range, scalability	[15]
Prime Editing	Very High	Very Low	Correcting complex mutations	Complex delivery, scalability challenges	[15]

Figure 2 displays various CRISPR-Cas9 delivery techniques and formats for gene editing. On the left, distribution methods include viral systems adenovirus (AV), adeno-associated virus (AAV) and lentivirus (LV), non-viral systems (lipids, polymers, and peptides), and physical approaches (electroporation and microinjection). On the right, CRISPR-Cas9 can be administered as Cas9 ribonucleoprotein (RNP), Cas9 mRNA with sgRNA, or plasmid DNA. These delivery techniques aid in the carriage of gene-editing components into cells, where Cas9 breaks the target DNA sequence to enable precise genetic change.

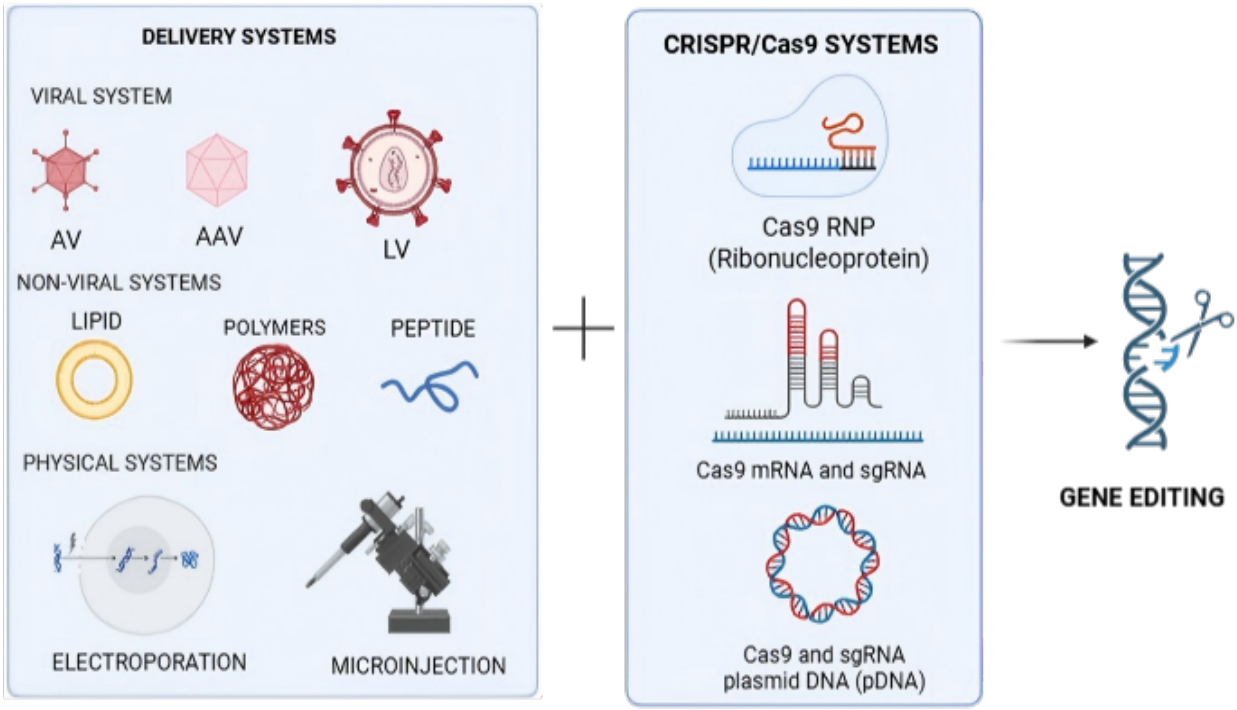

Figure 2. Delivery strategies for CRISPR-Cas9-based gene therapy.

3.6 Case Studies and Preclinical Research

Along with clinical trials, preclinical studies have used animal models and have shown that CRISPR-Cas9 has been able to correct genetic mutations without any problems. In cystic fibrosis, the Cas9 developed by CRISPR-Cas9 is now used to correct the Cystic Fibrosis Transmembrane Conductance Regulator gene, causing the pathology, which in turn has shown the ability to conduct proper ion transportation in cells. This encouraging preclinical data indicates that CRISPR-Cas9-based gene therapy may not just be effective in haematologically based disease but may also apply to disease processes of other body systems, such as the lungs and the muscles [16]. In monogenic illnesses like cystic fibrosis and Duchenne muscular dystrophy, CRISPR-mediated repair of disease-causing mutations in cell and animal models has resulted in gene function restoration, better cellular physiology, and long-term phenotype rescue. Preclinical editing of haematopoietic stem cells targeting foetal HbF expression regulators, particularly the BCL11A erythroid enhancer, has shown robust and durable therapeutic effects in haematological disorders, particularly SCD and β-thalassemia. This has directly informed the design of successful clinical trials [17].

Beyond inherited diseases, CRISPR-Cas9 has shown promise in preclinical cancer and immunotherapy research. Immune cells, including human T lymphocytes that are genetically modified by gene editing as well as other immune effector cells which are engineered to have increased anti-cancer activity or to be insensitive to immune checkpoint inhibition, have demonstrated better tumour clearance in animal models. The achievement in animal models is the reason for the start of the first in human clinical trials. These examples from actual cases demonstrate how versatile the CRISPR-Cas9 technology can be as a therapeutic platform and at the same time, they emphasize the importance of preclinical studies in optimizing the editing techniques, making them safe, and thus, opening the road to clinical applications [18].

4. Delivery Strategies for CRISPR-Based Gene Therapy

One of the most difficult hurdles in therapeutic genome editing is delivering CRISPR components efficiently and specifically to the right tissues. The safe transport of Cas proteins, guide RNAs, or messenger RNA into target cells while avoiding immune activation and off-target exposure is required for successful therapeutic applications. Delivery techniques are divided into three categories: viral, non-viral, and physical. Adeno-associated viral (AAV) vectors are commonly utilized in gene therapy experiments due to their high transduction efficiency and established safety profile. However, their low packaging capacity limits the delivery of big editing systems like prime editors, and prolonged Cas9 expression may increase off-target hazards [19]. Nonviral approaches, particularly lipid nanoparticles (LNPs), have shown promise for *in vivo* administration. LNP-mediated CRISPR delivery enables transient expression while reducing integration issues. This method has been successfully used in systemic in vivo editing approaches, such as transthyretin-targeted treatments. *Ex vivo* approaches use physical delivery methods, such as electroporation, to introduce CRISPR components into patient-derived cells under controlled settings before reinfusion. While this method improves editing precision and quality control, it necessitates specialized infrastructure and conditioning protocols. Despite significant advancements, important translational constraints continue to exist, including Cas protein immunogenicity, tissue selectivity, manufacturing scalability, and cost [20].

5. Challenges and Limitations

5.1 Ethical and Regulatory Concerns

There is a need for careful consideration of the ethical and regulatory aspects of CRISPR to ensure its responsible use as medicine, as CRISPR-Cas9 moves from preclinical research to standard clinical application. Besides the safety of the technology itself, there are also reflective moral concerns raised by gene editing about the safety of patients, the stability of the genome through time, germline alterations and fair access to advanced therapeutics [21].

The regulatory methodologies to CRISPR gene editing differ significantly among areas, as demonstrated in Table 2.

Table 2. Ethical and Regulatory frameworks governing CRISPR applications across regions [22].

Region	Regulation on Germline Editing	Focus of Research	Ethical Concerns
United States	Banned	Somatic gene editing	Designer babies, unintended consequences
European Union	Strictly prohibited	Somatic gene editing	Safety, equitable access
China	More permissive	Both somatic and germline editing	Safety concerns, regulatory transparency

5.2 Regional Regulatory Differences in CRISPR-Cas9

Common to all the countries is that they have all approached the regulation of CRISPR-Cas9 technology differently due to their differences in ethical, cultural, and scientific thinking. A slight analysis of CRISPR-Cas9 regulation in the United States, the European Union, and China is illuminating as to these differences [23].

In the United States, the federal government controls CRISPR-based research and therapy mostly through the Food and Drug Administration and the National Institutes of Health. Somatic gene editing, which alters non-reproductive cells but not gametes, is allowed in human research, as is enabling the work underpinning currently available clinical trials. Germline editing, however, is prohibited. Ethical issues are a matter of debate and future discussion of relaxing regulations, especially in somatic cell treatments, may reduce restrictions in the future. On the whole, the United States can be characterized by a comparatively liberal attitude toward CRISPR-Cas9 research, and ethical principles continue to be developed [23].

The European Union is more conservative in its way of action. The European Medicines Agency is the regulatory body of gene therapy in the European Union, and all germline editing is forbidden. The European Commission and member States lay much importance to ensuring safety and responsibility in the application of gene-editing technologies. As compared to the United States, where more research is conducted, the European Unionhas a tighter, stricter control over human gene editing. This involves stricter regulation of genetic therapy and the absence of agreement on the possible application of CRISPR-Cas9 to human germline modification [24].

China has already become a world leader in terms of conducting research with CRISPR-Cas9, enjoying a more relaxed policy than the United States and European Union, including the field of germline editing. The potential of CRISPR-Cas9 to edit human embryos and actual use cases have led to controversy and created legal issues when a Chinese scientist named He Jiankui edited embryos in 2018. Nonetheless, China has not stopped doing research on CRISPR, especially in areas of cancer treatment and other studies of genetic diseases. Since then, the government has issued more formal regulations in reaction to the controversy, but in general, the Chinese context of CRISPR-Cas9 research is freer as compared with the United States or European Union[25].

Despite the immense promise of CRISPR-Cas9 technology in gene therapy, ethical and regulatory questions are complex and not universally agreed upon, posing serious challenges to both the practical application of this technology and its market entry. On the ethical side of the issue, Figure 3 depicts the ethical framework for CRISPR-Cas9-mediated gene editing, which includes genomic change, regulatory control, and therapeutic application. The justice scale focuses on accountability and governance in therapeutic use. Importantly, these ethical values are inextricably linked to issues of access and equity, particularly in underdeveloped countries where affordability, infrastructure, and regulatory constraints may limit supply. Ensuring equitable distribution and global accessibility is thus critical for responsible implementation. For the global community to pursue more consistent regulation, cross-national liaisons must be maintained to deliver safe usage of the CRISPR-Cas9 technology [26].

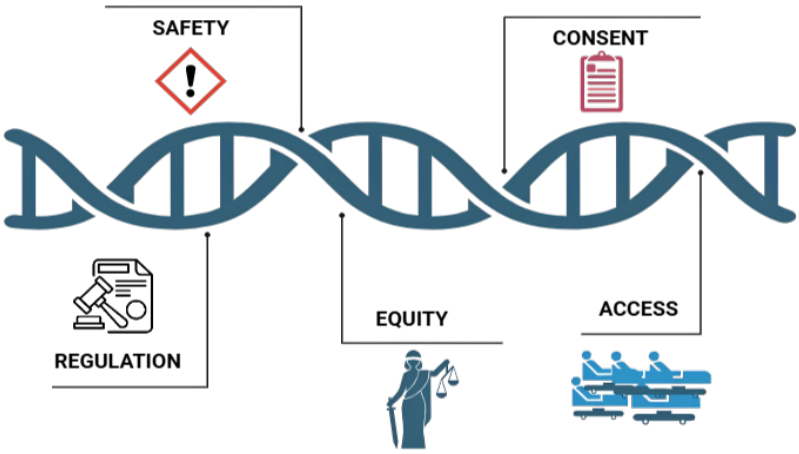

Figure 3. Ethical consideration in CRISPR-Cas9 mediated gene editing.

Figure 3 depicts the genome as a DNA double helix in the center. A clipboard denotes genetic analysis and target identification, while a justice scale represents ethical and regulatory oversight. Patients on hospital beds indicate clinical gene therapy uses. Overall, it connects genetic changes to research, ethics, and patient care.

6. Future Directions in CRISPR-Cas9 Gene Therapy

6.1 Expanding Therapeutic Applications

Although CRISPR-Cas9 is already yielding positive results in the treatment of genetic disorders, it can certainly be more broadly applied in other areas. Further studies will be aimed at using CRISPR-Cas9 technology in more complicated conditions like cancer, HIV, and neurological diseases. CRISPR-Cas9 can be used to edit T-cells to better target and kill cancer cells, or it can be used in cancer immunotherapy [27]. It is also possible to apply to Alzheimer's or other neurological cases by applying CRISPR-Cas9 to the edited genes that cause neurodegeneration. CRISPR-Cas9 technology can be used in precision medicine pipelines by combining patient-specific genomic analysis and targeted gene editing. This strategy enables medications to be adjusted to specific genetic variants, resulting in more effective

and personalised interventions. This integration not only enhances treatment specificity but also lowers the possibility of off-target effects, hence improving the safety and efficacy of CRISPR-based therapeutics [28].

6.2 Example of *In Vivo* Editing Trial: NTLA-2001

Intellia Therapeutics' NTLA-2001 is one of the first CRISPR-Cas9-based therapeutics designed for systemic genome editing in humans. NTLA-2001 targets the transthyretin gene in individuals with hereditary transthyretin amyloidosis, a rare and progressive disease caused by misfolded transthyretin protein buildup. Lipid nanoparticles carry CRISPR-Cas9 components intravenously to the liver, where transthyretin is generated. This results in targeted gene inactivation and sustained decrease of disease-causing proteins. Interim Phase 1 clinical data show rapid, dose-dependent, and long-lasting reductions in serum transthyretin protein after a single administration, with significant decreases observed at multiple dose levels and generally favorable safety and tolerability in early cohorts (e.g., up to ~87-96% mean transthyretin reduction by day). NTLA-2001's development demonstrates the possibility of *in vivo* CRISPR-Cas9 editing as a one-time, precision medicine that directly modifies disease-causing genes within the patient's body [29].

6.3 Improved Precision and New Technologies

With the growing technological and scientific advancements in CRISPR-Cas9 technology, this field is likely to be able to produce even more exacting tools of gene editing in the future. Base editing and prime editing are only the start. New CRISPR-Cas9 technologies, including CRISPR/Cas12 and CRISPR/Cas13 (which targets RNA), are being evaluated to treat several genetic diseases. Such developments will allow more precise control over the editing of genes, leading to a lower possibility of errors and widening the selection of treatable disorders [30].

In order to achieve greater precision and efficiency, the field of CRISPR-Cas9 research is increasingly utilizing machine learning strategies and artificial intelligence to achieve greater precision. These strategies enable the prediction of specificity, on-target efficiency, and minimize the possibility of off-target effects. These predictions enable the rational design of the guide RNA using the power of machine learning. Large amounts of genomic information are processed using complex computer models to identify the best sites to edit the genes. These models enable the unwanted changes to be predicted before they are actually carried out. The development of the next generation of CRISPR-based therapies is facilitated, the profile of these therapies is improved, and the time spent on trial and error is minimized. A crucial step towards the development of safe and accurate therapies lies in the integration of AI-based software into the field of genome editing [31].

6.4 *In Vivo* CRISPR-Cas9 Gene Editing

One of the most important frontiers of CRISPR-Cas9 gene therapy of the future is to develop in vivo CRISPR-Cas9 editing, that is, editing done inside the patient and not outside the patient (*ex vivo). In vivo* editing would not require complex processes such as cell extraction, alteration, and reinternment thereby simplifying the process and making it easier to use to patients. This can still be used to treat degenerative diseases and viral proteins, overcome obstruction in delivery, as well as precise targeting, but the application of this in the treatment of many diseases, even genetic diseases, cancerous, and viral infections, is potentially set to be a game-changer. Recent studies are working on safe and efficient delivery method as nanoparticles or viral vectors, in new ways, which will broaden *in vivo* editing and its clinical uses [32].

6.5 Personalized Medicine and CRISPR-Cas9

In the future, CRISPR-Cas9 may be applied in personalised medicine, where treatment can be customised to the patient with a specific genetic makeup. Individual mutations will also be analysed to enable the development of better, more personalized treatments using CRISPR-Cas9 because more specific therapies can be designed to eliminate the cause of a disease directly. This strategy will come in particularly handy in the case of more complicated diseases, where there are few treatment methods available [33].

With the advancement in genomic sequencing and molecular diagnostics, the use of CRISPR-Cas9 technology could have a significant influence on the possibilities that can be created pertaining to the treatment offered within the field of precision medicine. It may have applications beyond the treatment provided for genetic disorders, taking into consideration the treatment offered within the field of personalized medicine, leading towards new methods for treatment that could be provided for each patient [34].

6.6 Potential to Overcome Ethical and Regulatory Barriers

Even with the constant influx of scientific and technological discoveries, the integration of CRISPR-based drugs into everyday clinical use may largely hinge on the resolution of ethical and regulatory issues. On a global scale, scientists, doctors, bioethicists, and lawmakers should come together not only to establish a common ethical framework for gene-editing technologies but also for their application. Setting up international comprehensive guidelines, particularly for germline editing, could be of great help in overcoming the existing ethical concerns, securing the safety of patients, and enabling fair access to these medicines for the varied populations [35]. Besides, such frameworks would align safety

assessments, long-term monitoring, and risk management approaches, which in turn would raise the trust of the public in the technology. Additionally, it will be necessary for regulatory bodies, medical practitioners, and members of society to have frequent interactions so that innovation does not outpace societal values, potential new ethical problems can be identified, and policies can be adapted to the changes in the industry. Clinically, CRISPR-Cas9 can be extensively and universally employed if ethical management, regulatory scrutiny, and scientific innovation triumph together, hence cutting down the number of moral dilemmas and scaling up the level of patient benefit [36].

7. Conclusion

There is much hope here as the development of CRISPR-Cas9 continues to move forward with its application in gene therapy, supported by clinical evidence and preclinical data. The effectiveness that CRISPR-Cas9 has demonstrated regarding target specificity for the target DNA is what gives us hope for the treatment of genetic disorders. More automation within the editing process, as well as delivery, will unleash a whole new potential regarding the use of CRISPR-Cas9.

In the end, the potential of CRISPR-Cas9 may be one of the main technologies used in personalized medicine, being capable of successfully treating the complex disease of cancer or neurological disorders. It may bring a revolution in medical sciences if it is significantly improved soon, eradicating infections of genetically inherited illnesses, which have been untreated for a long time.

Even with the cons, which include off-target concerns, ethical issues, and delivery issues, CRISPR-Cas9 is still a revolution in gene therapy. The future of gene-editing technology rests on the current research, plus appropriate ethics, leading to a new era in genetic therapy.

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Conflict of Interest

The authors declare no conflict of interest.

Generative AI Statement

The Authors declare that no Gen AI was used in the creation of this manuscript.

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