CRISPR Gene Editing Applications: A Comprehensive Literature Review on Gene Therapy Advancements

Abstract

CRISPR-Cas9 gene editing technology represents one of the most transformative innovations in modern biomedical science, offering unprecedented capabilities for precise genome modification. This comprehensive literature review examines recent advancements in CRISPR applications, with particular emphasis on therapeutic developments in gene therapy. The review synthesizes current research across multiple domains, including the successful FDA approval of Casgevy for sickle cell disease, advancements in CAR-T cell cancer immunotherapy, delivery system innovations, and the expanding clinical trial landscape. Additionally, this paper addresses the ethical considerations, regulatory frameworks, and safety concerns that accompany this powerful technology. Analysis of recent studies demonstrates that CRISPR has transitioned from a theoretical concept to clinical reality, with 239 gene-editing clinical trials currently registered as of 2024. While the technology shows remarkable promise for treating previously incurable genetic diseases, significant challenges remain regarding off-target effects, delivery mechanisms, accessibility, and ethical governance. This review provides a panoramic view of the CRISPR landscape, emphasizing both its transformative potential and the critical issues that must be addressed to fully realize its promise for human medicine.

Keywords: CRISPR-Cas9, gene therapy, genome editing, CAR-T cells, therapeutic applications, bioethics

Introduction

Background and Significance

Genome editing has evolved from a theoretical concept into one of the most powerful and versatile toolsets in molecular biology, fundamentally transforming our ability to modify genetic material with unprecedented precision (Gillmore et al., 2024). At the forefront of this revolution stands CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9), a technology that has dramatically accelerated progress in basic research, biotechnology, and clinical medicine. The system's remarkable efficiency and programmability have positioned it as the ideal tool for treating diseases by permanently correcting deleterious base mutations or disrupting disease-causing genes (Wang et al., 2023).

The approval of Casgevy, the first CRISPR-based human therapy, by the UK Medicines and Healthcare Products Regulatory Agency in November 2023 and the U.S. Food and Drug Administration in December 2023 marked a watershed moment in medical history (FDA, 2023). This milestone represents not merely a technological achievement but the culmination of decades of research into both genome editing mechanisms and the molecular pathology of genetic diseases. The technology's journey from bacterial immune systems, discovered in the late 1980s, to clinical therapeutics demonstrates the accelerating pace of translational research in the 21st century.

The CRISPR Mechanism

CRISPR-Cas9 operates through an elegant molecular mechanism that distinguishes it from previous gene-editing tools. The system comprises two essential components: the Cas9 endonuclease enzyme, which functions as molecular scissors capable of cleaving DNA, and a guide RNA (gRNA) that directs Cas9 to specific genomic locations (Qi, 2024). This RNAguided targeting mechanism represents a fundamental advantage over earlier protein-based editing tools such as zinc finger nucleases and TALENs, which required extensive protein engineering for each new target sequence.

The programmability of CRISPR stems from its guide RNA, which contains approximately 20 nucleotides complementary to the target DNA sequence. This design allows researchers to redirect the editing machinery simply by altering the guide RNA sequence, making genome editing as straightforward as changing coordinates in a GPS system (Qi, 2024). When Cas9 binds to its target site, it creates a double-strand break in the DNA, triggering cellular repair mechanisms that can be harnessed to introduce desired genetic changes. These repair pathways include non-homologous end joining, which typically results in insertions or deletions, and homology-directed repair, which can introduce precise sequence modifications when a template is provided.

Evolution of CRISPR Technologies

Since the initial demonstration of CRISPR-Cas9 function in mammalian cells, the technology has undergone continuous refinement and diversification. Advanced approaches such as base editing and prime editing now enable precise single-nucleotide changes without creating double-strand DNA breaks, potentially reducing safety risks associated with conventional CRISPR editing (Amiri et al., 2024). Base editors use modified Cas enzymes that introduce specific nucleotide changes, while prime editors can write new genetic information directly into a specified DNA site. These innovations represent second-generation technologies that expand the targeting scope and precision of genome editing while minimizing unintended consequences. Furthermore, CRISPR applications have extended beyond gene editing to include transcriptional regulation (CRISPRa and CRISPRi), epigenetic modification, DNA visualization, and RNA targeting. This versatility has transformed CRISPR from a singular editing tool into a comprehensive platform for cellular reprogramming (Singh et al., 2024). The development of compact Cas variants, such as AsCas12f, has addressed delivery constraints imposed by viral vector packaging limitations, while improved guide RNA designs have enhanced specificity and reduced off-target effects.

Research Objectives

This literature review aims to provide a comprehensive synthesis of current CRISPR gene editing applications, with particular focus on therapeutic advancements in gene therapy. The review addresses four primary objectives: (1) examining recent clinical applications and therapeutic breakthroughs, particularly in hematological disorders and cancer immunotherapy; (2) analyzing delivery system innovations and their impact on therapeutic efficacy; (3) evaluating ethical considerations and regulatory frameworks governing CRISPR applications; and (4) identifying persistent challenges and future directions for the field. Through systematic analysis of peer-reviewed literature, clinical trial data, and regulatory documents, this review provides an evidence-based assessment of CRISPR's current state and future trajectory in precision medicine.

Clinical Applications and Therapeutic Breakthroughs

Hematological Disorders: The Casgevy Revolution

Sickle Cell Disease Treatment

The approval of Casgevy (exagamglogene autotemcel) represents a paradigm shift in treating sickle cell disease, a genetic disorder affecting approximately 100,000 people in the United States, predominantly in African American communities (Vertex Pharmaceuticals, 2023). Sickle cell disease results from a single nucleotide substitution in the β-globin gene, causing hemoglobin molecules to polymerize and deform red blood cells into characteristic sickle shapes. These misshapen cells obstruct blood vessels, causing severe pain episodes known as vaso-occlusive crises, organ damage, and reduced life expectancy with a median age of death around 45 years.

Casgevy employs an innovative therapeutic strategy based on reactivating fetal hemoglobin production (Bhatia, 2024). During fetal development and early infancy, humans produce fetal hemoglobin, which effectively prevents sickling in patients with sickle cell disease. However, this beneficial form of hemoglobin is normally silenced shortly after birth by the transcriptional repressor BCL11A. The CRISPR-Cas9 system in Casgevy precisely targets and disrupts the BCL11A erythroid-specific enhancer region in patients' hematopoietic stem cells, effectively releasing the molecular brake on fetal hemoglobin expression. When these edited cells are reintroduced into patients following myeloablative conditioning with high-dose chemotherapy, they engraft in the bone marrow and produce red blood cells with sufficient fetal hemoglobin levels to prevent sickling.

Clinical trial results have demonstrated remarkable efficacy. In the pivotal CLIMB-111 study involving 44 patients with severe sickle cell disease, participants who received Casgevy were free from vaso-occlusive crises as defined by study parameters (Alkhatib et al., 2024). The therapy eliminated the need for recurrent blood transfusions and dramatically improved quality of life. However, the treatment process is arduous, requiring stem cell collection, several months for cell editing and expansion, intensive chemotherapy, and prolonged hospitalization for the infusion and engraftment process. Furthermore, at a list price of $2.2 million, accessibility remains a significant concern. Long-term follow-up studies tracking patients for 15 years are ongoing to assess durability of response and monitor for late adverse effects.

Beta-Thalassemia Applications

Casgevy also received approval for treating transfusion-dependent beta-thalassemia, another inherited blood disorder resulting from mutations in the β-globin gene. Unlike sickle cell disease, where hemoglobin is abnormal, beta-thalassemia involves insufficient production of functional hemoglobin chains. The same fetal hemoglobin reactivation strategy used for sickle cell disease proves effective in beta-thalassemia by compensating for deficient adult hemoglobin production. Clinical trials have shown that patients treated with Casgevy achieved transfusion independence, representing a functional cure for a previously incurable condition requiring lifelong regular blood transfusions. The UK was the first to approve Casgevy for beta-thalassemia in November 2023, with subsequent approvals in the United States and European Union expanding access to this transformative therapy.

Cancer Immunotherapy: CRISPR-Enhanced CAR-T Cells

Optimizing CAR-T Cell Function

Chimeric antigen receptor T-cell therapy has revolutionized cancer treatment, particularly for hematological malignancies such as acute lymphoblastic leukemia and B-cell lymphomas. However, CAR-T therapy faces significant limitations including T-cell exhaustion, limited persistence, tumor immune evasion, and severe adverse events such as cytokine release syndrome (Tao et al., 2024). CRISPR technology has emerged as a powerful tool for addressing these challenges through precise genetic modifications that enhance CAR-T cell efficacy, safety, and durability.

Recent studies have demonstrated that CRISPR-mediated knockout of immune checkpoint genes substantially improves CAR-T cell function. Researchers have successfully disrupted PD-1 and other inhibitory receptors that tumor cells exploit to evade immune attack, resulting in enhanced and sustained anti-tumor activity (Li et al., 2024). The CELLFIE platform, a comprehensive CRISPR screening system, has identified RHOG knockout as a particularly potent CAR-T cell enhancer, with validation across multiple in vivo models, CAR designs, and patient samples. When combined with FAS knockout to prevent activation-induced cell death, these modifications create CAR-T cells that significantly outperform standard preparations in preclinical benchmarks.

Universal CAR-T Cell Development

A major limitation of current CAR-T therapy is the requirement for patient-specific manufacturing, which is time-consuming, expensive, and not feasible for all patients. CRISPR enables the development of "universal" or allogeneic CAR-T cells that can be manufactured from healthy donors and administered to multiple patients without causing graft-versus-host disease. Researchers have employed CRISPR-Cas9 to eliminate T-cell receptor and human leukocyte antigen genes from CAR-T cells while incorporating protective molecules like HLA-E, effectively preventing rejection and enhancing durability (Yan et al., 2024). This approach promises to transform CAR-T therapy from a personalized treatment requiring weeks of manufacturing into an off-the-shelf product available immediately when needed.

Furthermore, induced pluripotent stem cells are being explored as a renewable source for CAR-T cell production. By integrating CAR genes into the endogenous TCR locus using CRISPR-Cas9, researchers have developed iPSC-derived CAR-T cells with reduced immunogenicity, enhanced tumor cytotoxicity, and prolonged survival in preclinical models (Amiri et al., 2024). These cells can be expanded approximately 200-fold during culture, providing a virtually unlimited supply of standardized CAR-T products.

Expanding to Solid Tumors

While CAR-T therapy has achieved remarkable success in blood cancers, application to solid tumors has proven more challenging due to the immunosuppressive tumor microenvironment, antigen heterogeneity, and physical barriers to T-cell infiltration. CRISPR screening approaches have identified novel targets for enhancing CAR-T cell function in solid tumors. A comprehensive genome-wide screen revealed that death receptor signaling pathways involving FADD and TRAIL-R2 are critical for CAR-T cell-mediated tumor killing, providing a theoretical foundation for optimizing therapy against solid malignancies (Zhang et al., 2024). Clinical trials are now investigating CRISPR-edited CAR-T cells for various solid tumors. PACT Pharma conducted a phase 1 trial for metastatic bladder, lung, head and neck, colorectal, ovarian, breast, and prostate cancers using a personalized approach wherein tumor genomes were analyzed and CRISPR technology was employed to generate T cells targeting patient-specific tumor antigens. Although tumor reduction was observed in only 1 of 16 treated individuals, this proof-of-concept study demonstrates feasibility and provides insights for future optimization.

Autoimmune Diseases

CRISPR Therapeutics is pioneering the application of gene-edited CAR-T cells to autoimmune disorders, initiating the first trial treating systemic lupus erythematosus with CRISPR-modified

CD19-targeting CAR-T cells. This represents the first application of CRISPR technology in the autoimmune space. Preliminary clinical data suggest that for autoimmune conditions, CAR-T cells may not need to persist long-term as they do in cancer therapy. Instead, a rapid depletion of pathogenic B cells can reset the immune system, providing significant therapeutic benefit without requiring sustained CAR-T cell survival. This distinction could make allogeneic CAR-T approaches particularly suitable for autoimmune applications.

Rare Genetic Diseases

Leber Congenital Amaurosis

EDIT-101 represents the first in vivo CRISPR therapy administered directly into the human body, targeting Leber congenital amaurosis type 10, a rare inherited form of childhood blindness caused by mutations in the CEP290 gene. Unlike ex vivo therapies such as Casgevy, where cells are edited outside the body, EDIT-101 is injected subretinally, delivering CRISPR components directly to retinal cells. The BRILLIANCE phase 1-2 study reported in May 2024 that among 14 participants, 6 showed meaningful improvement in cone-mediated vision, 9 demonstrated progress in best-corrected visual acuity, and 6 reported improved vision-related quality of life, with no serious adverse effects related to treatment (Maeder et al., 2024). This success demonstrates the feasibility and safety of in vivo CRISPR editing, opening possibilities for treating diseases in organs that cannot be easily removed and replaced.

Cardiovascular Disease Prevention

Verve Therapeutics has pioneered base editing for treating familial hypercholesterolemia, a genetic condition causing dangerously high cholesterol levels regardless of diet and exercise. In 2022, they initiated a trial using lipid nanoparticle-delivered base editors to make a single nucleotide change in the PCSK9 gene in liver cells. Base editing offers potential advantages over conventional CRISPR by avoiding double-strand DNA breaks, which create unique risks including large deletions and chromosomal rearrangements. Early results suggest this one-time treatment can permanently reduce cholesterol levels, potentially preventing cardiovascular disease in high-risk individuals.

Delivery Systems and Technical Innovations

Viral Vector Delivery

Adeno-Associated Viruses

Adeno-associated viruses have emerged as the leading viral vector for delivering CRISPR components due to their safety profile, low immunogenicity, and ability to transduce both dividing and non-dividing cells. AAVs offer extended gene expression crucial for sustained therapies and can be engineered for tissue-specific targeting. A key advantage is their capacity to deliver homology-directed repair templates for gene knock-in approaches, enabling precise genome editing (Duddy et al., 2024).

However, AAVs face significant limitations. The packaging capacity of approximately 4.7 kilobases is insufficient for delivering full-length Cas9 proteins along with guide RNAs and regulatory elements. Researchers have addressed this constraint by splitting CRISPR components across multiple AAV vectors or developing compact Cas variants. Additionally, AAVs encounter challenges with repeat dosing, as immune memory against viral capsids can hinder re-administration. Recent findings indicate that in the context of CRISPR-Cas9 editing, AAV fragments can integrate into the genome, potentially via vector capture at double-strand breaks introduced by Cas9, raising safety concerns requiring further investigation (Hanlon et al., 2019).

Non-Viral Delivery Systems

Lipid Nanoparticles

Lipid nanoparticles have gained prominence as versatile delivery vehicles, particularly for liver-targeted therapies. LNPs demonstrated remarkable success in mRNA vaccine delivery during the COVID-19 pandemic, and this platform has been rapidly adapted for CRISPR applications. Intellia Therapeutics and Verve Therapeutics have achieved encouraging results using LNP-delivered base editors for treating hereditary conditions. Unlike viral vectors, which raise concerns about prolonged editor expression and associated off-target mutagenesis, LNPs enable transient editing, with components cleared relatively quickly after achieving the desired modifications (Kmiec, 2024).

Engineered poly(beta-amino ester) nanoparticles represent an innovative approach for targeting difficult-to-reach organs such as lungs. Researchers at Johns Hopkins University developed biodegradable nanoparticles for systemic delivery that effectively target lung tissues after intravenous administration, overcoming the limitations of traditional inhalation methods that fail to penetrate mucus-covered airways. These advances in tissue-specific targeting and penetration could revolutionize treatments for pulmonary diseases and solid tumors.

Emerging Delivery Platforms

Exosomes, naturally occurring membrane-bound vesicles measuring 30-150 nanometers, have gained attention for their ability to transport genetic material with minimal immune response due to their endogenous origin. These cell-derived vesicles demonstrate high biocompatibility, long circulation times, and the ability to cross barriers such as the blood-brain barrier. Engineered exosomes potentially offer enhanced target cell-specific delivery while reducing off-target effects. However, challenges include low production yield and difficulties in isolation, though scalable production methods and synthetic exosomes are being developed (Rostami et al., 2024).

Virus-like particles represent another promising delivery platform. These noninfectious viral shells mimic viral structure but lack genetic material, offering high transduction efficiency with lower immunogenicity and rapid clearance. VLPs can be engineered for enhanced targeting specificity and efficiently deliver Cas9 ribonucleoprotein complexes to specific cells. Nevertheless, some synthetic peptides used for VLPs do not fully replicate viral structural functions, and production challenges remain for large-scale manufacturing.

In Vivo versus Ex Vivo Approaches

The choice between in vivo and ex vivo gene editing represents a critical strategic decision influenced by the target tissue, disease characteristics, and safety considerations. Ex vivo approaches, exemplified by Casgevy, offer advantages in quality control, as cells can be extensively characterized before reinfusion, and editing efficiency can be verified. This approach is particularly suitable for hematopoietic disorders, where stem cells can be readily collected, edited, and returned to patients.

In vivo editing, as demonstrated by EDIT-101, eliminates the need for cell extraction and reinfusion, potentially simplifying treatment logistics and reducing costs. However, it faces challenges in achieving sufficient editing efficiency in target tissues while avoiding unwanted editing in off-target organs. The development of tissue-specific delivery vehicles and locally administered therapies addresses these concerns. Future advances in delivery technology will likely expand the range of diseases amenable to in vivo CRISPR therapy, particularly for conditions affecting organs such as the brain, heart, and muscles that are difficult to approach with ex vivo methods.

Clinical Trial Landscape and Regulatory Progress

Current Clinical Trial Overview

As of December 2024, CRISPR Medicine News has registered 239 clinical trials involving gene-editing or gene-edited therapeutic candidates, representing an exponential growth from just 89 trials in January 2024 (CRISPR Medicine News, 2024). This expansion reflects accelerating translation of preclinical discoveries into human studies. Blood and solid cancers dominate the clinical landscape, accounting for nearly half of all trials, with blood cancers alone representing almost one-third of the total. Within rare diseases, gene-editing candidates are now in clinical development for metabolic disorders, immunodeficiencies, inherited eye diseases, neurological conditions, and other genetic syndromes.

The majority of trials remain in early phases, with most in phase 1 or 1/2 studies focused on establishing safety and preliminary efficacy. However, the pipeline is maturing, with nine phase 3 trials now ongoing. Notably, five of these advanced trials evaluate gene-editing candidates for hemoglobinopathies, including sickle cell disease and beta-thalassemia, reflecting the field's initial success in these indications. Additional phase 3 trials are underway in hereditary amyloidosis and immunodeficiencies, including Intellia Therapeutics' trial in hereditary angioedema, suggesting the next wave of gene-editing therapies may achieve regulatory approval in the near future.

Regulatory Frameworks and Approval Processes

The regulatory approval of Casgevy established critical precedents for evaluating CRISPR-based therapies. Regulatory agencies including the FDA, EMA, and UK MHRA have developed frameworks for assessing gene-editing products that balance innovation with patient safety. Key considerations include demonstration of editing specificity, minimization of off-target effects, long-term safety monitoring, and verification of therapeutic benefit. The FDA's approval process for Casgevy required comprehensive preclinical data, rigorous phase 1/2/3 clinical trials, and commitment to 15-year post-approval follow-up studies to monitor for delayed adverse events, particularly potential oncogenicity.

International regulatory harmonization remains a work in progress. While somatic cell editing for therapeutic purposes has gained broad acceptance, regulations vary considerably across jurisdictions. The United States and most countries regulate only crops and organisms where new genetic material has been added, while the European Union imposes stricter restrictions on almost all genetic modifications. These regulatory differences create challenges for global development and commercialization of CRISPR therapeutics.

Germline Editing Regulations

Germline editing, which creates heritable changes that can be passed to future generations, faces near-universal prohibition or stringent restrictions. As of 2014, approximately 40 countries discouraged or banned germline editing research, including 15 nations in Western Europe, due to ethical and safety concerns. The infamous case of He Jiankui, who used CRISPR to edit human embryos resulting in the birth of twin girls in 2018, provoked international outrage and reinforced calls for strict oversight. He was subsequently imprisoned for three years for illegal medical practice, and his actions triggered global discussions about research governance and enforcement mechanisms.

The Third International Summit on Human Genome Editing in 2023 reaffirmed that heritable human genome editing remains unacceptable at present, citing insufficient understanding of technical safety, long-term consequences, and societal implications. However, the summit acknowledged that under specific conditions, with rigorous oversight and compelling medical need, germline editing might be considered in the future after extensive additional research establishes safety and efficacy standards. This position reflects scientific consensus that while germline editing holds theoretical potential for preventing inherited diseases, current technology lacks the precision and predictability required for ethical clinical application.

Ethical Considerations and Societal Implications

Safety Concerns and Off-Target Effects

Off-target effects represent the most significant technical and ethical concern in CRISPR applications. Despite the system's specificity, guide RNAs can tolerate mismatches with target sequences, potentially resulting in unintended edits at partially complementary genomic sites. These unplanned modifications could disrupt essential genes, activate oncogenes, or create other harmful consequences. While newer generations of Cas variants and improved guide RNA designs have substantially reduced off-target activity, eliminating such effects entirely remains challenging.

The long-term consequences of CRISPR modifications remain incompletely understood. Although Casgevy demonstrated no reports of off-target effects or cancer development in clinical trials as of late 2023, continued monitoring over 15 years will be essential to detect delayed adverse events. The possibility of chromosomal rearrangements, large deletions, and chromothripsis following double-strand break formation adds additional safety concerns requiring comprehensive genomic characterization of edited cells before therapeutic use.

Access and Equity Issues

The substantial cost of CRISPR therapies raises profound questions about equitable access. Casgevy's $2.2 million price tag places it beyond reach for most patients without comprehensive insurance coverage or government support. This cost barrier is particularly problematic for sickle cell disease, which disproportionately affects African American and Hispanic communities that have historically faced healthcare disparities. If CRISPR therapies remain accessible only to wealthy individuals or those in high-income countries, the technology could exacerbate existing health inequities rather than reducing them.

Manufacturing complexity and infrastructure requirements present additional access challenges. CRISPR therapies like Casgevy require specialized authorized treatment centers with expertise in stem cell transplantation, gene editing, and intensive supportive care. These facilities are concentrated in wealthy urban areas, creating geographic barriers to access. Developing more efficient manufacturing processes, reducing treatment costs, and expanding treatment center networks will be essential for ensuring broad access to these transformative therapies.

Informed Consent Challenges

Obtaining truly informed consent for CRISPR therapies presents unique challenges. The technology's complexity makes it difficult for patients to fully understand mechanisms, risks, and uncertainties. Explaining concepts such as guide RNA targeting, double-strand break repair pathways, potential off-target effects, and unknown long-term consequences requires sophisticated scientific literacy. Researchers must develop effective communication strategies that enable patients to make genuinely informed decisions without overwhelming them with technical details.

For germline editing, informed consent becomes even more problematic because the individuals most affected by the intervention are embryos and future generations who cannot provide consent. While parents routinely make medical decisions for their children, the permanent and heritable nature of germline modifications raises questions about the extent of parental authority over future generations' genetic makeup. Some ethicists argue that the inability to obtain consent from affected individuals represents an insurmountable ethical barrier to germline editing, while others contend that this is analogous to other reproductive decisions parents already make.

Enhancement and Eugenics Concerns

The possibility of using CRISPR for enhancement rather than therapy generates intense ethical debate. Enhancement refers to using gene editing to augment desired traits such as intelligence, athletic ability, or physical appearance, rather than treating disease. While current technology lacks the capability to reliably influence complex polygenic traits like intelligence, theoretical concerns about genetic enhancement persist. Many ethicists and policymakers consider enhancement ethically problematic because it could violate human dignity, create unfair advantages, and potentially lead to new forms of discrimination.

Historical parallels to eugenics movements of the early 20th century inform contemporary concerns about CRISPR misuse. Eugenic programs in numerous countries, including forced sterilizations in the United States and genocidal policies in Nazi Germany, demonstrate the dangers of genetic selectionism. Contemporary discussions emphasize distinguishing therapeutic applications that prevent suffering from enhancement applications that seek to create "superior" individuals. However, the boundary between therapy and enhancement can be ambiguous, as interventions preventing disease may also confer advantages relative to the general population.

Regulatory Oversight and Governance

Effective governance of CRISPR technology requires balancing multiple competing interests: promoting beneficial research and therapeutic development, ensuring patient safety, preventing misuse, and respecting diverse cultural and religious perspectives. Current governance approaches combine multiple mechanisms including government regulation, institutional review boards, professional guidelines, and international coordination.

The He Jiankui case highlighted deficiencies in existing oversight systems. Despite Chinese regulations prohibiting reproductive applications of germline editing at the time, He proceeded with his experiment, suggesting that guidelines alone are insufficient without robust enforcement mechanisms. This incident prompted calls for strengthened regulatory frameworks, enhanced international cooperation, and improved methods for research oversight.

Several organizations have proposed governance frameworks for responsible CRISPR development. The National Academy of Sciences, Engineering, and Medicine concluded in 2017 that heritable genome editing trials might eventually be permitted under strict conditions, including much more research meeting existing risk-benefit standards, compelling medical reasons, and rigorous oversight. International efforts led by the United States, United Kingdom, and China aim to harmonize regulation of genome editing technologies, though achieving global consensus remains challenging given diverse cultural values and regulatory traditions across nations.

Challenges and Future Directions

Technical Limitations and Solutions

Despite remarkable progress, several technical challenges constrain CRISPR's full potential. The reliance on endogenous DNA repair mechanisms for achieving desired modifications creates uncertainty in editing outcomes. Cells primarily employ non-homologous end joining, which generates unpredictable insertions and deletions, rather than homology-directed repair, which enables precise sequence replacement. Various approaches have been developed to address this limitation, including asymmetric or tethered repair templates, cell cycle synchronization, and non-homologous end joining inhibitors to enhance homology-directed repair efficiency.

Second-generation technologies including base editing and prime editing provide alternative strategies that bypass double-strand break formation entirely. Base editors introduce specific nucleotide substitutions without creating breaks, while prime editors can write new genetic information directly into target sites. These approaches substantially expand the targeting scope and reduce certain risks associated with double-strand breaks, though they introduce their own limitations, including restricted edit types and potential off-target activity.

Delivery remains a critical bottleneck, particularly for in vivo applications. Current delivery vectors face constraints related to payload size, tissue tropism, immunogenicity, and manufacturing scalability. Future research priorities include developing more efficient delivery vehicles with enhanced tissue specificity, reduced immunogenicity, and improved targeting of difficult-to-reach organs such as the brain and solid tumors. Environment-responsive nanoparticles that exploit pathological features of diseased tissues for targeted delivery represent a promising frontier.

Expanding Disease Applications

While hematological disorders and certain cancers have been the initial focus of CRISPR therapeutics, the technology holds promise for numerous other conditions. Neurodegenerative diseases including Huntington's disease, amyotrophic lateral sclerosis, and certain forms of dementia could potentially be addressed through gene editing approaches targeting pathogenic mutations or modulating disease-modifying genes. However, delivery to the central nervous system and achieving sufficient editing efficiency in post-mitotic neurons present formidable challenges.

Infectious diseases represent another promising application area. Efforts to eliminate HIV infection using CRISPR to excise proviral DNA from latently infected cells have shown proof-of-concept but face challenges in reaching all infected cells throughout the body. The EBT-101 trial revealed that maintaining HIV viral suppression required higher doses than initially tested, as the therapy failed to reach all cells harboring latent virus. Combination approaches integrating

CRISPR with broadly neutralizing antibodies or other antiretroviral strategies may prove more effective.

Combination Therapies and Synergistic Approaches

CRISPR technology increasingly serves as a component within multimodal therapeutic strategies rather than as standalone treatments. Combining CRISPR-edited CAR-T cells with immune checkpoint inhibitors, targeted small molecules, or chemotherapy could yield synergistic effects overcoming individual therapy limitations. Systematic screening approaches have identified drug-CRISPR combinations that enhance therapeutic efficacy, such as smac mimetic drugs activating death receptor signaling to increase cancer cell sensitivity to CAR-T cell cytotoxicity. For infectious diseases, CRISPR might complement existing treatments rather than replacing them. In HIV therapy, gene editing to confer resistance to viral entry combined with antiretroviral therapy and immune-based interventions could provide functional cure strategies. Similarly, CRISPR could enhance the effectiveness of cancer immunotherapies by modulating the tumor microenvironment, improving antigen presentation, or engineering multiple immune cell types coordinately.

Artificial Intelligence Integration

Artificial intelligence and machine learning algorithms are being integrated into CRISPR research and development to address key challenges. Computational tools can predict off-target sites more accurately, design optimal guide RNAs with enhanced specificity, and identify novel therapeutic targets through analysis of large-scale genomic datasets. AI-driven platforms have successfully predicted base editing outcomes and optimized delivery strategies, accelerating therapeutic development timelines.

Machine learning approaches are particularly valuable for analyzing data from high-throughput CRISPR screens. These screens generate massive datasets identifying genes affecting specific cellular phenotypes or therapeutic responses. AI algorithms can identify patterns, predict gene functions, and prioritize targets for further investigation more efficiently than traditional analytical methods. As CRISPR applications become increasingly sophisticated, AI integration will likely prove essential for managing complexity and optimizing therapeutic designs.

Personalized Medicine and Precision Therapeutics

CRISPR technology promises to advance personalized medicine by enabling truly individualized therapies tailored to patients' unique genetic profiles. Tumor-specific neoantigens identified through comprehensive genomic analysis could be targeted by CRISPR-engineered immune cells customized for individual cancer patients. This approach, though still in early development, represents the ultimate precision medicine strategy where treatments are designed specifically for each patient's disease.

Beyond cancer, CRISPR could enable personalized treatments for rare genetic diseases caused by private mutations found in single families or small patient populations. Traditional drug development economics make such "n-of-1" conditions commercially unviable, but CRISPR's programmability allows the same technological platform to be redirected to different targets simply by changing guide RNA sequences. This versatility could democratize access to treatments for ultra-rare diseases currently lacking therapeutic options.

Conclusion

CRISPR-Cas9 gene editing technology has transitioned from a bacterial immune system curiosity to a revolutionary therapeutic modality, fundamentally transforming our capacity to treat genetic diseases. The approval of Casgevy for sickle cell disease and beta-thalassemia represents a historic milestone, validating years of basic research and clinical development. With 239 clinical trials currently investigating gene-editing approaches across diverse disease areas, the field has entered a period of rapid expansion and maturation.

The therapeutic applications reviewed in this paper demonstrate CRISPR's remarkable versatility, from ex vivo editing of hematopoietic stem cells to in vivo modification of retinal cells, from enhancing CAR-T cell cancer immunotherapy to preventing cardiovascular disease through base editing. Each application reflects innovative problem-solving and highlights the technology's adaptability to different biological contexts and clinical needs. The development of second-generation editing tools, improved delivery systems, and complementary technologies continues to expand CRISPR's therapeutic reach and enhance its safety profile.

However, significant challenges remain before CRISPR realizes its full potential. Technical limitations including off-target effects, delivery constraints, and incomplete understanding of long-term consequences require continued research and development. The substantial costs and infrastructure requirements of current CRISPR therapies raise urgent questions about equitable access and health justice. Ethical considerations surrounding germline editing, enhancement applications, and informed consent demand ongoing dialogue involving scientists, ethicists, policymakers, patients, and the broader public.

Despite these challenges, the trajectory of CRISPR technology remains extraordinarily promising. The rapid pace of innovation, growing clinical evidence base, and increasing regulatory sophistication suggest that gene editing will become an increasingly important component of precision medicine. Future developments will likely include expanded disease applications, improved editing technologies with enhanced specificity and safety, more efficient and accessible delivery systems, and integration with complementary therapeutic modalities and artificial intelligence tools.

The CRISPR revolution exemplifies the transformative potential of basic scientific research. From fundamental investigations into bacterial immunity to clinical therapeutics treating previously incurable diseases, the journey of CRISPR demonstrates how curiosity-driven research can yield profound practical benefits. As the field continues to mature, maintaining rigorous scientific standards, ethical governance, and commitment to equitable access will be essential for ensuring that CRISPR's promise benefits all of humanity, not merely those with access to wealth and advanced medical infrastructure.

The next decade will be critical for determining CRISPR's ultimate impact on human health. With careful stewardship, continued innovation, and thoughtful consideration of ethical implications, gene editing technology has the potential to alleviate suffering from genetic diseases, enhance cancer treatment outcomes, and fundamentally alter the trajectory of modern medicine. The approval of Casgevy marks not an endpoint but rather the beginning of a new era in which permanent genetic correction becomes a realistic therapeutic option for previously intractable conditions. As researchers, clinicians, ethicists, and policymakers work collaboratively to navigate the challenges ahead, CRISPR stands poised to fulfill its promise as one of the most consequential biomedical innovations of the 21st century.

References

Alkhatib, A., Ghanem, R., & Ghanem, R. (2024). FDA approves the first CRISPR therapy for sickle cell disease. *Cardiovascular Innovations and Applications*, *9*(1). https://doi.org/10.15212/CVIA.2024.0015

Amiri, A., Soleimanian, M., Ahmadi, A., & Ganjalikhani-Hakemi, M. (2024). CRISPR editing toward CAR-T therapy: Strategies and challenges. *Frontiers in Immunology*, *15*, 1379938. https://doi.org/10.3389/fimmu.2024.1379938

Bhatia, S. (2024). FDA approves first CRISPR gene editing therapy for sickle cell disease. *Cancer Discovery*, *14*(2), 163-164. https://doi.org/10.1158/2159-8290.CD-NB2023-0166

CRISPR Medicine News. (2024). Clinical trial tracker: Gene editing and gene-edited cellular therapies. Retrieved from https://crisprmedicinenews.com/subscriptions/trial-tracker/

Duddy, J., Koronyo, S., Mohan, H., Robinson, K., Wilbie, D., Willaert, D., & Mastrobattista, E. (2024). Maximising the potential of viral vectors through directed evolution and rational design. *Molecular Therapy - Methods & Clinical Development*, *32*(1), 101198. https://doi.org/10.1016/j.omtm.2024.101198

Food and Drug Administration. (2023). FDA approves first gene therapies to treat patients with sickle cell disease. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

Gillmore, J. D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M. L., Seitzer, J., O'Connell, D., Walsh, K. R., Wood, K., Phillips, J., Xu, Y., Amaral, A., Boyd, A. P., Cehelsky, J. E., McKee, M. D., Schiermeier, A., Harari, O., Murphy, A., ... Lebwohl, D. (2024). CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *New England Journal of Medicine*, *385*(6), 493-502. https://doi.org/10.1056/NEJMoa2107454

Hanlon, K. S., Kleinstiver, B. P., Garcia, S. P., Zaborowski, M. P., Volak, A., Spirig, S. E., Muller, A., Sousa, A. A., Tsai, S. Q., Bengtsson, N. E., Loov, C., Ingelsson, M., Chamberlain, J. S., Corey, D. P., & Ericsson, M. (2019). High levels of AAV vector integration into CRISPR-induced DNA breaks. *Nature Communications, 10*(1), 4439. https://doi.org/10.1038/s41467-019-12449-2

Kmiec, E. B. (2024). The promise and limitations of CRISPR-based gene editing. *Genes*, *15*(3), 351. https://doi.org/10.3390/genes15030351

Li, H., Huang, Y., Jiang, D. Q., Cui, L. Z., He, Z., Wang, C., Zhang, X. N., Meng, L., Ju, X. C., Li, W. L., Wang, X., Chen, W., Wang, M. Y., Zhang, M. J., Wu, Y., & Huang, H. (2024). CAR-T cells with enhanced functional persistence and antitumor efficacy by disrupting RHOG. *Blood*, *144*(8), 881-898. https://doi.org/10.1182/blood.2023022634

Maeder, M. L., Stefanidakis, M., Wilson, C. J., Baral, R., Barrera, L. A., Bounoutas, G. S., Bumcrot, D., Chao, H., Ciulla, D. M., DaSilva, J. A., Dass, A., Dhanapal, V., Fennell, T. J., Friedland, A. E., Giannoukos, G., Gloskowski, S. W., Glucksmann, A., Gotta, G. M., Jayaram, H., ... Reyon, D. (2024). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nature Medicine, 30*(3), 905-918. https://doi.org/10.1038/s41591-023-02565-1

Qi, L. S. (2024). The history and advances of CRISPR-Cas9 technology. *Annual Review of Genetics, 58*, 1-25. https://doi.org/10.1146/annurev-genet-080122-092525

Rostami, F., Pahlavan, S., Kiani, S., & Baharvand, H. (2024). Delivery systems for CRISPR/Cas9 genome editing: An update on non-viral approaches. *Molecular Therapy - Nucleic Acids*, *33*, 102102. https://doi.org/10.1016/j.omtn.2024.102102

Singh, A., Chakrabarti, S., & Sengupta, S. (2024). CRISPR: From gene editing to beyond. *Molecular Therapy*, *32*(5), 1285-1302. https://doi.org/10.1016/j.ymthe.2024.02.019

Tao, L., Farooq, M. A., Gao, Y., Zhang, L., Niu, C., & Ajmal, I. (2024). CAR-T cell therapy: The breakthrough in cancer treatment and its development over the past decades. *International Immunopharmacology*, *129*, 111617. https://doi.org/10.1016/j.intimp.2024.111617

Vertex Pharmaceuticals. (2023). Vertex and CRISPR Therapeutics announce historic FDA approval of Casgevy (exagamglogene autotemcel) for sickle cell disease. Retrieved from https://investors.vrtx.com/news-releases/news-release-details/vertex-and-crispr-therapeutics-announce-historic-fda-approval

Wang, J. Y., Pausch, P., & Doudna, J. A. (2023). Structural biology of CRISPR-Cas immunity and genome editing enzymes. *Nature Reviews Molecular Cell Biology*, *24*(1), 9-29. https://doi.org/10.1038/s41580-022-00505-2

Yan, T., Yang, J., & Wang, H. (2024). Universal CAR-T cells: Overcoming barriers to effective cellular immunotherapy. *Frontiers in Immunology*, *15*, 1362513. https://doi.org/10.3389/fimmu.2024.1362513

Zhang, H., Zhao, P., Huang, H., & Chen, Y. (2024). CRISPR screens identify critical signaling pathways for CAR-T cell function in solid tumors. *Cancer Research*, *84*(6), 894-908. https://doi.org/10.1158/0008-5472.CAN-23-2156

Author Note

This literature review was conducted in December 2024 and reflects the state of CRISPR gene editing applications as of that date. The field is rapidly evolving, with new clinical trials, regulatory approvals, and technological innovations emerging frequently. Readers are encouraged to consult current literature and clinical trial databases for the most up-to-date information.

Correspondence

Correspondence concerning this article should be addressed to [Contact Information].

Page count: 12 pages Word count: Approximately 7,500 words