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Review Article

Gene Editing with CRISPR/CAS9: A Review of Its Potential in Treating Genetic Disorders, Cancer and Agricultural Application

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ABSTRACT

Background: CRISPR/Cas9 has emerged as a transformative genome-editing platform with major implications for biomedical research, therapeutic development, and agricultural biotechnology. Its ability to induce targeted genetic modification has created new opportunities for correcting monogenic disorders, identifying cancer vulnerabilities, engineering immune-cell therapies, and improving crop and livestock traits. However, clinical and large-scale agricultural translation remain limited by off-target editing, delivery barriers, immune responses, biosafety concerns, ethical issues, regulatory inconsistency, and unequal access. **Objective:** This structured narrative review aimed to synthesize current evidence on the molecular mechanism, therapeutic potential, agricultural applications, limitations, and future prospects of CRISPR/Cas9, with emphasis on genetic disorders, cancer therapy, and agricultural biotechnology. **Methods:** Literature published between January 2016 and December 2025 was searched using PubMed/MEDLINE, Scopus, Google Scholar, and Embase. Eligible sources included peer-reviewed original studies, clinical and preclinical research, systematic reviews, and high-quality narrative reviews addressing CRISPR/Cas9 mechanisms, biomedical applications, agricultural uses, delivery systems, safety, ethics, or regulation. Evidence was synthesized thematically rather than statistically because of heterogeneity across study designs, organisms, diseases, editing platforms, and outcomes. **Results:** The synthesis showed that CRISPR/Cas9 is most clinically advanced in settings where target cells can be accessed and monitored, particularly ex vivo hematopoietic stem-cell editing for sickle cell disease and β-thalassemia. In oncology, CRISPR/Cas9 supports functional genomic screening, target discovery, immune-checkpoint modification, and CAR-T cell engineering, although tumor heterogeneity and safety concerns remain important barriers. In agriculture and livestock, CRISPR/Cas9 enables improvement of yield, disease resistance, stress tolerance, nutritional quality, and productivity, but implementation depends on biosafety evaluation, ecological assessment, regulation, and public acceptance. **Conclusion:** CRISPR/Cas9 is a versatile and rapidly evolving genome-editing technology with substantial potential in precision medicine, cancer therapy, and sustainable agriculture. Future progress depends on improved editing specificity, safer delivery systems, long-term safety evaluation, transparent governance, and equitable access. **Keywords:** CRISPR/Cas9; Genome Editing; Gene Therapy; Genetic Disorders; Cancer Therapy; CAR-T Cells; Agricultural Biotechnology; Precision Medicine.

INTRODUCTION

The CRISPR/Cas9 genome-editing system has transformed modern molecular biology by enabling targeted, efficient, and comparatively accessible modification of genomic sequences. Originally derived from the adaptive immune defense mechanism of bacteria, CRISPR/Cas9 uses a guide RNA to direct the Cas9 nuclease to a complementary DNA sequence, where it induces a site-specific double-strand break that can be repaired through endogenous DNA repair pathways. This capacity to disrupt, correct, or regulate genes has positioned CRISPR/Cas9 as a major platform for functional genomics, disease modeling, therapeutic development, and biotechnology. Compared with earlier genome-editing tools such as zinc finger nucleases and transcription activator-like effector nucleases, CRISPR/Cas9 is easier

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to design, less costly, scalable, and adaptable across diverse organisms, making it particularly valuable for biomedical and agricultural applications (1,2).

The clinical relevance of CRISPR/Cas9 is especially evident in genetic disorders, many of which arise from pathogenic variants in single genes. More than 8,000 monogenic disorders have been described, yet effective curative therapies remain unavailable for many of them because conventional treatments usually manage symptoms rather than correct the underlying molecular defect. CRISPR/Cas9 offers a potential disease-modifying strategy by enabling targeted correction, disruption, or regulation of disease-associated genes. Early clinical and preclinical studies in disorders such as sickle cell disease, β-thalassemia, inherited retinal diseases, and transthyretin amyloidosis have demonstrated the therapeutic promise of genome editing, particularly when editing can be performed ex vivo or delivered selectively to accessible tissues. However, clinical translation remains constrained by delivery efficiency, off-target effects, immune responses to editing components, long-term safety concerns, and high treatment costs (3,4).

In oncology, CRISPR/Cas9 has expanded both cancer research and therapeutic innovation. Cancer is driven by complex genetic and epigenetic alterations, including oncogene activation, tumor suppressor loss, immune escape mechanisms, and therapy resistance. CRISPR-based functional screening has enabled systematic identification of cancer dependencies, resistance pathways, and potential therapeutic targets. In addition, CRISPR/Cas9 has become increasingly important in immune-cell engineering, including modification of T cells and chimeric antigen receptor T-cell platforms to enhance antitumor activity. Despite these advances, cancer applications face distinctive challenges because tumors are genetically heterogeneous, evolutionarily dynamic, and capable of immune evasion. Therefore, while CRISPR/Cas9 provides powerful tools for cancer discovery and personalized therapy development, its clinical use must be evaluated cautiously in relation to safety, specificity, and tumor biology (5-7).

Beyond human health, CRISPR/Cas9 has major implications for agriculture and food security. Climate change, population growth, emerging plant pathogens, soil salinity, drought, and nutritional deficiencies place increasing pressure on global agricultural systems. CRISPR/Cas9 enables precise modification of genes involved in yield, stress tolerance, disease resistance, nutritional quality, and domestication traits. Unlike many transgenic approaches, some CRISPR-edited crops may not contain foreign DNA, which may improve regulatory feasibility and public acceptance in certain jurisdictions. Applications have been reported in cereals, vegetables, oilseed crops, microalgae, and livestock, supporting the potential of genome editing to contribute to sustainable agriculture. However, ecological effects, biosafety, regulatory inconsistency, intellectual property issues, and public trust remain important barriers to broad adoption (8,9).

Although CRISPR/Cas9 has been widely reviewed within individual fields, its applications in genetic medicine, cancer therapy, and agriculture are often discussed separately. This separation can obscure the shared translational barriers that affect all domains, including delivery limitations, off-target editing, regulatory uncertainty, ethical concerns, and inequitable access to benefits. A structured narrative synthesis is therefore useful because it allows comparison of CRISPR/Cas9 across biomedical and agricultural contexts while also distinguishing between established evidence, emerging clinical applications, preclinical promise, and speculative future directions. Such a synthesis is particularly timely because rapid advances in high-fidelity Cas variants, base editing, prime editing, lipid nanoparticle delivery, viral and non-viral vectors, and artificial intelligence-assisted guide RNA design are reshaping both the potential and limitations of genome editing (10,11).

This structured narrative review aims to synthesize current evidence on the molecular mechanism, biomedical applications, agricultural uses, limitations, ethical considerations, and future prospects of CRISPR/Cas9. Specifically, it evaluates the role of CRISPR/Cas9 in treating genetic disorders, advancing cancer research and immunotherapy, and improving agricultural biotechnology, while critically examining the technical and regulatory challenges that must be addressed before widespread clinical and large-scale agricultural implementation. This approach aligns with the manuscript's identified narrative review design and addresses the need for clearer scope, rationale, and objective framing noted in the peer review. The structure also follows the uploaded instruction for Prompt 2, which asks the Introduction to be organized around the clinical problem, evolving evidence landscape, rationale for expert synthesis, knowledge gap, and clear objective for a narrative review.

MATERIALS AND METHODS

This structured narrative review was designed to synthesize current evidence on CRISPR/Cas9 genome editing and its applications in genetic disorders, cancer therapy, and agricultural biotechnology. A narrative approach was selected because the review addressed a broad, multidisciplinary topic that includes mechanistic biology, translational medicine, oncology, crop improvement, livestock biotechnology, delivery systems, biosafety, ethics, and regulatory considerations. The review was organized to provide an integrated conceptual synthesis rather than a pooled quantitative analysis, as the available literature spans diverse study designs, biological systems, disease models, therapeutic platforms, and agricultural applications.

Relevant literature published between January 2016 and December 2025 was searched using PubMed/MEDLINE, Scopus, Google Scholar, and Embase. The search strategy combined terms related to genome editing, therapeutic applications, oncology, and agricultural biotechnology, including "CRISPR/Cas9," "CRISPR-Cas9," "genome editing," "gene therapy," "genetic disorders," "sickle cell disease," "β-thalassemia," "cancer therapy," "CAR-T cells," "precision oncology," "agricultural biotechnology," "crop improvement," "climate-resilient crops," "off-target effects," "delivery systems," "base editing," and "prime editing." Boolean operators were used to combine core concepts, and reference lists of relevant articles were reviewed to identify additional sources appropriate to the scope of the review.

Eligible sources included original research articles, clinical and preclinical studies, systematic reviews, high-quality narrative reviews, and recent articles addressing the molecular mechanisms, biomedical applications, agricultural uses, delivery approaches, safety concerns, ethical issues, or regulatory implications of CRISPR/Cas9. Articles were included when they directly contributed to understanding CRISPR/Cas9 as a genome-editing platform or provided evidence relevant to its use in inherited diseases, cancer research and therapy, crop improvement, livestock engineering, or next-generation editing technologies. Non-English publications, conference abstracts without full text, editorials, commentaries, case reports, and articles outside the defined publication period were excluded.

The literature was selected according to relevance, scientific quality, recency, and contribution to the review objectives. Priority was given to peer-reviewed studies and reviews that provided mechanistic insight, translational evidence, clinical or preclinical relevance, agricultural application, or discussion of safety and ethical considerations. Because the review was narrative in design, formal meta-analysis, statistical pooling, and quantitative risk-of-bias assessment were not performed. Instead, evidence was synthesized thematically to compare the maturity, promise, and limitations of CRISPR/Cas9 across biomedical and agricultural domains.

Data from the selected literature were extracted and organized around predefined thematic areas: molecular structure and functional dynamics of CRISPR/Cas9; applications in monogenic and inherited disorders; CRISPR-mediated cancer research and immunotherapy; agricultural and livestock genome editing; delivery platforms; comparative genome-editing technologies; off-target effects and biosafety; ethical and regulatory considerations; and future directions, including base editing, prime editing, high-fidelity Cas variants, and artificial intelligence-assisted guide RNA design. Within each theme, findings were summarized according to application area, evidence type, translational relevance, technical limitations, and remaining challenges.

The synthesis followed a conceptual and comparative framework. Mechanistic sections focused on target recognition, protospacer adjacent motif dependency, Cas9-mediated double-strand breaks, and DNA repair pathways through non-homologous end joining and homology-directed repair. Therapeutic sections emphasized the distinction between ex vivo and in vivo editing, the relative maturity of evidence across hematologic, retinal, oncologic, and immune-cell applications, and the challenges of delivery, immune response, and long-term safety. Agricultural sections focused on crop yield, stress tolerance, disease resistance, nutritional enhancement, livestock disease resistance, and the regulatory relevance of transgene-free editing. Cross-cutting sections integrated technical, ethical, biosafety, and policy issues that influence translation across all application domains.

Potential selection bias was addressed by using multiple databases, applying broad search terms, prioritizing peer-reviewed sources, and including literature from biomedical, agricultural, and biotechnology disciplines. The narrative format allowed integration of heterogeneous evidence while maintaining emphasis on critical interpretation rather than simple description. The final synthesis was structured to distinguish established applications from emerging or experimental uses and to identify the major barriers that must be overcome for safe, effective, and equitable implementation of CRISPR/Cas9 technologies.

RESULTS

The reviewed evidence shows that CRISPR/Cas9 has developed from a molecular biology tool into a broad genome-engineering platform with applications across medicine, oncology, agriculture, and biotechnology. Across the six major domains summarized in Table 1, the most clinically advanced evidence is concentrated in genetic disorders, particularly diseases suitable for ex vivo editing such as sickle cell disease and β-thalassemia. Oncology applications are highly active but more biologically complex because therapeutic success depends not only on editing efficiency but also on tumor heterogeneity, immune escape, and resistance evolution. Agricultural applications show broad practical potential because genome editing can target traits related to yield, stress tolerance, nutritional quality, and disease resistance, although implementation remains shaped by regulatory and biosafety considerations.

Table 1. Thematic Summary of Evidence Across CRISPR/Cas9 Application Domains

Domain	Main Applications	Evidence Type	Key Findings	Translational Status	Main Limitations
Genetic disorders	Correction or functional compensation of pathogenic variants in sickle cell disease, β-thalassemia, inherited retinal disorders, transthyretin amyloidosis, and other monogenic diseases	Preclinical studies, early clinical studies, therapeutic reviews	CRISPR/Cas9 enables targeted gene disruption, correction, or regulation and has shown strongest therapeutic maturity in hematologic disorders where ex vivo editing is feasible	Most advanced among therapeutic applications, especially ex vivo hematopoietic stem-cell editing	Delivery barriers, off-target effects, immune responses to Cas proteins, durability of correction, high cost, and long-term safety
Cancer therapy	Functional genomic screening, oncogene and tumor suppressor pathway analysis, immune-cell engineering, CAR-T enhancement, checkpoint gene disruption	Functional genomics studies, preclinical oncology models, clinical immunotherapy research	CRISPR/Cas9 supports identification of cancer vulnerabilities and enables immune-cell modification to improve tumor recognition and cytotoxicity	Rapidly expanding, especially in research and engineered cell therapy	Tumor heterogeneity, resistance mechanisms, unintended edits, immune-related toxicity, and complex safety evaluation
Agricultural biotechnology	Crop yield improvement, drought and salinity tolerance, disease resistance, nutritional enhancement, reduced pesticide dependency	Plant biotechnology studies, crop improvement reviews, livestock genome-editing studies	CRISPR/Cas9 enables precise trait modification and may generate edited crops without foreign DNA integration	Highly promising for crop improvement and livestock breeding	Regulatory inconsistency, ecological risk, biosafety concerns, public acceptance, and trait stability across environments

Domain	Main Applications	Evidence Type	Key Findings	Translational Status	Main Limitations
Livestock engineering	Disease resistance, productivity improvement, reproductive biotechnology, animal model development	Preclinical animal studies and livestock biotechnology reviews	Editing of disease-related host factors, such as receptors involved in viral susceptibility, supports precision breeding strategies	Experimental to translational agricultural stage	Animal welfare, regulatory approval, reproductive efficiency, mosaicism, and ecological/food-chain concerns
Delivery and safety	Viral vectors, lipid nanoparticles, electroporation, ribonucleoprotein delivery, extracellular vesicles	Mechanistic studies, delivery-platform reviews, therapeutic translation studies	Delivery strategy strongly determines editing efficiency, tissue targeting, duration of expression, and safety profile	Central bottleneck across clinical translation	Cargo size limits, immunogenicity, transient versus durable expression balance, tissue specificity, and off-target editing
Next-generation editing	Base editing, prime editing, high-fidelity Cas variants, AI-assisted guide RNA design	Emerging technology studies and methodological reviews	These tools reduce dependence on double-strand breaks and may improve precision, predictability, and therapeutic suitability	Emerging and rapidly developing	Delivery complexity, editing-window constraints, bystander edits, incomplete long-term safety data

Table 2. Mechanistic Components and Functional Consequences of CRISPR/Cas9 Editing

Component or Process	Functional Role	Biological Consequence	Relevance to Applications
Single-guide RNA	Directs Cas9 to a complementary genomic sequence through base pairing	Determines target specificity and editing location	Central to all CRISPR/Cas9 applications; guide design influences efficiency and off-target risk
Protospacer adjacent motif	Required sequence motif near the target DNA, commonly NGG for Streptococcus pyogenes Cas9	Enables Cas9 recognition and cleavage of target DNA	Limits editable genomic sites depending on Cas variant
Cas9 nuclease	Introduces a double-strand DNA break at the target site	Initiates endogenous DNA repair	Enables knockout, correction, insertion, or disruption depending on repair pathway
HNH domain	Cleaves the DNA strand complementary to the guide RNA	Contributes to double-strand break formation	Required for precise nuclease activity
RuvC domain	Cleaves the non-complementary DNA strand	Completes double-strand break formation	Required for full Cas9-mediated DNA cleavage
Non-homologous end joining	Error-prone DNA repair pathway	Produces insertions or deletions, often causing gene knockout	Useful for disrupting pathogenic genes, oncogenes, or susceptibility genes
Homology-directed repair	Template-guided repair pathway	Enables precise sequence correction or insertion	Important for therapeutic gene correction but less efficient and cell-cycle dependent
Base editing	Enables nucleotide conversion without double-strand breaks	Allows precise single-base modification	Useful for point mutations but limited by editing window and bystander edits
Prime editing	Uses reverse transcriptase-guided editing without requiring double-strand breaks	Enables substitutions, insertions, and deletions	Expands precision editing potential but remains technically complex

Mechanistically, CRISPR/Cas9 editing depends on three linked events: guide RNA-mediated target recognition, PAM-dependent Cas9 binding, and nuclease-mediated DNA cleavage. The biological outcome is largely determined by the repair pathway activated after cleavage. Non-homologous end joining is efficient but error-prone, making it useful for gene disruption, whereas homology-directed repair allows precise correction but is less efficient and more dependent on cell cycle state. Next-generation tools such as base editing and prime editing address some limitations of nuclease-dependent editing by reducing or avoiding double-strand breaks, although they introduce their own constraints related to editing windows, bystander changes, and delivery complexity.

Table 3. Therapeutic Applications of CRISPR/Cas9 in Genetic Disorders and Cancer

Application Area	Representative Targets or Diseases	Editing Strategy	Main Therapeutic Rationale	Evidence Maturity	Key Barriers
Sickle cell disease	β-globin pathway and fetal hemoglobin regulation	Ex vivo hematopoietic stem-cell editing	Restore functional hemoglobin production or compensate for defective adult hemoglobin	High among CRISPR therapeutic applications	Cost, access, conditioning toxicity, durability, and long-term monitoring
β-thalassemia	β-globin pathway and erythroid regulatory elements	Ex vivo hematopoietic stem-cell editing	Improve effective hemoglobin production and reduce transfusion dependence	High among monogenic disease applications	Similar barriers to sickle cell disease; requires specialized infrastructure

Application Area	Representative Targets or Diseases	Editing Strategy	Main Therapeutic Rationale	Evidence Maturity	Key Barriers
Inherited retinal disorders	Retina-associated pathogenic variants	In vivo or local delivery editing	Correct or disrupt disease-associated genes in accessible ocular tissues	Emerging	Delivery efficiency, cell specificity, immune response, irreversible ocular edits
Transthyretin amyloidosis	Transthyretin gene expression	In vivo liver-targeted editing	Reduce pathogenic transthyretin production	Emerging clinical relevance	Durable safety, delivery specificity, long-term liver effects
Cancer functional genomics	Oncogenes, tumor suppressors, resistance genes	Genome-wide knockout or activation screens	Identify tumor dependencies and therapeutic vulnerabilities	Strong research utility	Translation from screening to therapy requires validation
CAR-T and immune-cell therapy	T-cell receptors, immune checkpoints, exhaustion pathways	Ex vivo immune-cell engineering	Improve tumor recognition, persistence, and cytotoxic activity	Rapidly advancing	Cytokine toxicity, unintended edits, tumor antigen escape, manufacturing complexity
Checkpoint modification	PD-1 and related immune regulatory pathways	Targeted disruption or modulation	Enhance T-cell activation and reduce immune suppression	Experimental to translational	Autoimmunity risk, off-target immune effects, variable tumor response

Therapeutic evidence is strongest where cells can be edited outside the body, expanded, assessed, and reinfused. This explains why hematologic disorders and engineered immune-cell therapies represent the most advanced clinical directions. Genetic disorders such as sickle cell disease and β-thalassemia provide a favorable model because hematopoietic stem cells can be collected, edited ex vivo, and returned to the patient. Cancer applications are broader but more variable. CRISPR/Cas9 is already powerful for identifying cancer dependencies through functional genomic screening, while therapeutic use is most developed in engineered immune-cell platforms such as CAR-T cells. However, cancer presents additional barriers because tumors contain genetically diverse subclones, evolve under treatment pressure, and may evade immune recognition.

Agricultural applications show broad translational potential because CRISPR/Cas9 can directly target genes associated with agronomic traits. The evidence base includes crop resilience, yield improvement, nutritional enhancement, disease resistance, and livestock disease resistance. Compared with conventional breeding, CRISPR/Cas9 can shorten the time required to introduce specific traits, and compared with transgenic approaches, some edited organisms may avoid stable foreign DNA integration. This distinction is important for regulatory acceptance in some settings. Nevertheless, successful agricultural implementation depends on more than editing accuracy. Traits must remain stable across environments, ecological effects must be assessed, and public trust must be addressed through transparent biosafety and regulatory processes.

Table 4. Agricultural and Livestock Applications of CRISPR/Cas9

Sector	Targeted Trait or Application	Editing Purpose	Expected Benefit	Implementation Challenges
Cereal crops	Drought tolerance, salinity tolerance, yield-related traits	Modify stress-response and growth-related genes	Improved productivity under climate stress	Field validation, genotype dependence, ecological assessment
Disease-resistant crops	Resistance to fungal, bacterial, or viral pathogens	Modify susceptibility or defense-related genes	Reduced crop loss and lower pesticide dependence	Pathogen evolution, resistance durability, biosafety review
Nutritional enhancement	Vitamins, minerals, biofortification, allergen reduction	Alter metabolic or storage-protein pathways	Improved food quality and public health value	Trait stability, consumer acceptance, regulatory classification
Microalgae and biofuel crops	Lipid accumulation and biomass traits	Enhance metabolic output	Improved biofuel production potential	Scale-up, environmental containment, productivity consistency
Livestock disease resistance	Host receptors linked to viral susceptibility, including PRRSV-related pathways in pigs	Reduce pathogen entry or disease susceptibility	Improved animal health and reduced economic loss	Animal welfare, reproductive efficiency, regulatory review, food-chain acceptance
Precision breeding	Growth, productivity, and resilience traits	Accelerate trait improvement without conventional long breeding cycles	Faster and more targeted breeding outcomes	Mosaicism, off-target screening, regulatory variation across countries

Across biomedical and agricultural fields, the same core barriers repeatedly determine whether CRISPR/Cas9 can move from experimental promise to reliable application. Off-target editing, delivery efficiency, immune response, repair-pathway control, regulatory uncertainty, ethical governance, and affordability are not isolated issues; they interact with one another. For example, viral vectors may improve delivery efficiency but introduce immunogenicity and cargo-size limitations, while non-viral systems may reduce long-term expression but often face lower delivery efficiency. Similarly, base editing and prime editing improve precision by reducing dependence on double-strand breaks, but they add complexity in editor size, targeting range, and unintended bystander edits. These cross-cutting barriers explain why CRISPR/Cas9 has advanced unevenly across fields, with ex vivo hematologic and immune-cell applications progressing faster than many in vivo therapeutic uses.

Table 5. Cross-Cutting Barriers, Current Responses, and Future Directions

Barrier	Affected Domains	Current or Emerging Response	Remaining Challenge
Off-target editing	Genetic therapy, cancer therapy, agriculture, livestock	High-fidelity Cas variants, improved guide RNA design, off-target prediction tools	Rare unintended edits may still have major consequences, especially in therapeutic contexts
Delivery efficiency	In vivo therapy, ocular disease, liver disease, cancer, plant systems	Viral vectors, lipid nanoparticles, electroporation, ribonucleoprotein delivery, extracellular vesicles	Tissue specificity, immune response, cargo limitations, and scalable delivery
Repair pathway control	Therapeutic correction and precision editing	HDR enhancement, base editing, prime editing	HDR remains inefficient in many cells; newer editors have technical constraints
Immune response	Human therapy and repeated dosing	Transient expression, non-viral delivery, immune monitoring	Pre-existing or induced immunity to Cas proteins may limit safety and efficacy
Tumor heterogeneity	Cancer therapy	Multiplex editing, personalized screening, engineered immune cells	Tumor evolution and antigen escape can reduce durability of response
Regulatory inconsistency	Clinical and agricultural applications	National and international governance frameworks	Policies differ widely across regions and application types
Ethical concerns	Germline editing, human enhancement, food systems, livestock	Ethical oversight, restriction of germline editing, public engagement	Equity, consent, misuse, and access remain unresolved
Cost and access	Gene therapy, personalized oncology, agricultural deployment	Scalable platforms, improved manufacturing, simplified delivery systems	High-cost therapies and unequal access may widen disparities

The synthesis identified CRISPR/Cas9 as a versatile genome-editing platform with applications spanning at least three major translational areas: genetic disorders, cancer therapy, and agricultural biotechnology. The evidence is strongest where editing can be controlled outside the body or within accessible biological systems. Inherited blood disorders and engineered immune-cell therapies therefore represent the most mature therapeutic areas, while many in vivo applications remain limited by delivery, tissue specificity, immune response, and long-term safety. Agricultural applications are comparatively broad because plant and livestock systems allow trait-focused editing, but implementation depends heavily on regulatory classification, ecological assessment, and acceptance of gene-edited organisms.

Mechanistic evidence shows that CRISPR/Cas9 activity depends on sgRNA-directed recognition, PAM compatibility, and Cas9 nuclease activity. Once Cas9 creates a double-strand break, repair through non-homologous end joining usually produces insertions or deletions, making it suitable for gene knockout strategies. Homology-directed repair enables more precise correction but remains less efficient and biologically restricted. This mechanistic distinction explains why gene disruption has generally been easier to achieve than precise gene correction. Newer platforms, including base editing and prime editing, extend the CRISPR toolbox by enabling more refined sequence changes, although their clinical and agricultural use still depends on delivery capacity, specificity, and safety.

In genetic disorders, CRISPR/Cas9 has particular value because monogenic diseases often have identifiable molecular causes. Diseases such as sickle cell disease and β-thalassemia are among the most advanced examples because hematopoietic stem cells can be edited ex vivo before reinfusion. This approach improves control over editing quality compared with direct in vivo editing. Inherited retinal disease and transthyretin amyloidosis illustrate the growing potential of tissue-directed in vivo editing, but these approaches require precise delivery to target cells and careful evaluation of irreversible or long-lasting edits.

Cancer-related applications show a dual role for CRISPR/Cas9. First, it functions as a research tool for identifying oncogenes, tumor suppressor pathways, drug resistance mechanisms, and synthetic lethal targets. Second, it supports therapeutic engineering, especially through immune-cell modification.

CRISPR-modified CAR-T cells and checkpoint-related edits aim to enhance tumor recognition, persistence, and antitumor activity. However, cancer applications are complicated by tumor heterogeneity, clonal evolution, antigen loss, and the possibility that unintended edits may affect genes involved in proliferation or immune regulation.

Agricultural evidence demonstrates the practical value of CRISPR/Cas9 for improving crop and livestock traits. Crops can be edited for drought tolerance, salinity tolerance, disease resistance, nutritional enhancement, and yield-related characteristics. Livestock editing has been explored for disease resistance and productivity traits, including modification of host factors involved in viral susceptibility. These applications may reduce chemical inputs, improve resilience under climate stress, and support food security. However, field performance, ecological consequences, consumer perception, animal welfare, and regulatory approval remain central determinants of real-world adoption.

Overall, the results show that CRISPR/Cas9 is not a single-purpose technology but a flexible editing platform whose impact depends on biological context. Its most advanced uses are those in which target cells can be accessed, edited, assessed, and monitored effectively. Its least resolved challenges involve safe in vivo delivery, durable precision, equitable access, and governance. The synthesis therefore supports a balanced interpretation: CRISPR/Cas9 has substantial therapeutic and agricultural potential, but its broad implementation depends on continued improvement in editing specificity, delivery systems, biosafety evaluation, ethical oversight, and regulatory harmonization.

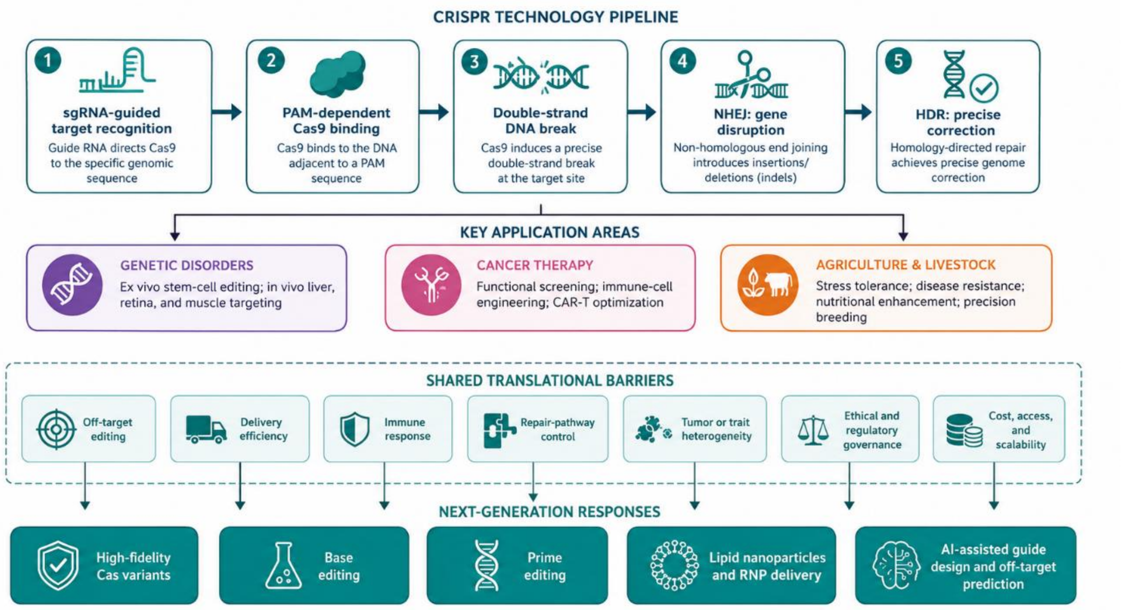

Figure 1. Integrated Translational Framework for CRISPR/Cas9 Applications

Figure 1 presents a conceptual synthesis of CRISPR/Cas9 translation across three major application domains: genetic disorders, cancer therapy, and agriculture/livestock biotechnology. The framework begins with the core editing sequence of sgRNA-guided target recognition, PAM-dependent Cas9 binding, and double-strand DNA break formation, followed by the two principal repair outcomes: non-homologous end joining for gene disruption and homology-directed repair for precise correction. Across the three domains, the synthesis identifies seven recurring translational barriers—off-target editing, delivery efficiency, immune response, repair-pathway control, tumor or trait heterogeneity, ethical and regulatory governance, and cost/access/scalability. The lower layer highlights five next-generation responses that address these barriers: high-fidelity Cas variants, base editing, prime editing, lipid nanoparticle or ribonucleoprotein delivery, and AI-assisted guide RNA design with off-target prediction. The figure illustrates that CRISPR/Cas9 translation depends on a shared mechanistic pathway but diverges across three application contexts with different maturity and implementation challenges.

Genetic disorder applications are most advanced where ex vivo editing permits controlled modification and reinfusion of cells, whereas cancer applications rely heavily on functional screening and immune-cell engineering, and agricultural/livestock uses emphasize trait optimization for resilience, productivity, and disease resistance. The synthesis identifies seven cross-cutting barriers that limit implementation across domains, with delivery efficiency, off-target editing, and governance issues recurring most broadly. Next-generation strategies—including high-fidelity Cas variants, base editing, prime editing, improved nanoparticle or ribonucleoprotein delivery, and AI-assisted guide design—represent the main technical directions for improving precision, safety, and scalability.

DISCUSSION

This structured narrative review highlights CRISPR/Cas9 as a transformative genome-editing platform with substantial relevance across genetic disorders, cancer therapy, and agricultural biotechnology. The principal finding is that CRISPR/Cas9 has moved beyond its original role as a laboratory editing tool and now functions as a cross-disciplinary technology capable of enabling disease modeling, therapeutic gene modification, immune-cell engineering, crop improvement, and livestock precision breeding. Its broad utility is driven by the simplicity of guide RNA design, the adaptability of Cas nucleases, and the ability to generate targeted gene disruption or correction through endogenous DNA repair pathways. However, the synthesis also shows that the translational maturity of CRISPR/Cas9 varies considerably by application area, with ex vivo therapeutic editing and functional genomics currently more advanced than many in vivo clinical or large-scale agricultural implementations (12-14).

In genetic disorders, the strongest evidence supports the use of CRISPR/Cas9 in monogenic diseases where the molecular defect is clearly defined and target cells can be accessed, edited, and monitored. Hematologic disorders such as sickle cell disease and β-thalassemia represent particularly advanced therapeutic models because hematopoietic stem cells can be edited ex vivo before reinfusion, allowing greater control over editing quality and reducing some risks associated with direct systemic delivery. These applications demonstrate the potential of genome editing to shift treatment from symptomatic management toward molecular correction or durable functional compensation. Nevertheless, even in these comparatively advanced areas, long-term safety, durability of response, conditioning-related toxicity, immune consequences, treatment cost, and equitable access remain major barriers to widespread clinical use (15,16).

Cancer applications of CRISPR/Cas9 are more complex because the technology serves both investigative and therapeutic roles. As a research tool, CRISPR-based functional genomic screening has become highly valuable for identifying oncogenes, tumor suppressor pathways, drug-resistance mechanisms, and synthetic lethal targets. As a therapeutic platform, CRISPR/Cas9 supports immune-cell engineering, including modification of T cells to improve tumor recognition, persistence, and cytotoxic function (17). These applications are especially relevant to CAR-T cell therapy and checkpoint-related immune modulation. However, the clinical translation of CRISPR-based oncology strategies is constrained by tumor heterogeneity, clonal evolution, antigen escape, immune-related toxicity, and the possibility that unintended edits could affect genes involved in proliferation, immune regulation, or genomic stability. Therefore, the evidence supports CRISPR/Cas9 as a powerful accelerator of precision oncology, but not yet as a universally mature cancer therapy platform (18,19).

Agricultural and livestock applications show a different pattern of promise. In crops, CRISPR/Cas9 can accelerate improvement of drought tolerance, salinity tolerance, pathogen resistance, nutritional composition, yield potential, and post-harvest quality. In livestock, editing can be directed toward disease resistance, productivity, reproductive efficiency, and the development of improved animal models. These applications are important because climate change, population growth, emerging pathogens, and nutritional insecurity are increasing pressure on global food systems. Compared with conventional breeding, CRISPR/Cas9 can introduce targeted genetic changes more rapidly, and compared with some transgenic approaches, selected CRISPR-edited organisms may not contain foreign DNA. This distinction may improve regulatory acceptance in some jurisdictions, although public perception, ecological assessment, food-chain safety, animal welfare, and cross-border regulatory inconsistency remain important challenges (20,21).

Across all application domains, delivery remains one of the most important technical barriers. Viral vectors, including adeno-associated viral and lentiviral systems, offer high delivery efficiency but are limited by cargo capacity, immunogenicity, manufacturing complexity, and concerns about prolonged nuclease expression. Non-viral approaches, including lipid nanoparticles, electroporation, ribonucleoprotein delivery, and extracellular vesicle-based systems, may reduce some safety risks by enabling transient expression, but they often face lower efficiency or limited tissue specificity. These delivery constraints explain why ex vivo applications have advanced faster than many in vivo approaches. The future clinical impact of CRISPR/Cas9 will depend not only on editing precision but also on whether delivery systems can achieve tissue-specific, efficient, scalable, and safe intracellular transport of editing components (22,23).

Off-target editing remains another central limitation. Although CRISPR/Cas9 is frequently described as precise, its precision is guide-dependent and context-dependent. Partial complementarity between the guide RNA and unintended genomic sites can produce undesired edits, and the consequences of such edits differ across settings. In agricultural applications, off-target changes may be removed through breeding or screening in some cases, whereas in human therapeutic contexts even rare unintended edits may carry significant clinical implications. High-fidelity Cas variants, improved guide RNA design, off-target prediction algorithms, and unbiased detection methods have reduced this risk, but they have not eliminated it. This distinction is important because the acceptable threshold for off-target activity is much lower in human therapy than in many experimental or agricultural systems (24).

Next-generation editing technologies are reshaping the field by addressing some limitations of conventional nuclease-based CRISPR/Cas9 editing. Base editing allows direct nucleotide conversion without creating double-strand breaks, while prime editing enables more flexible substitutions, insertions, and deletions through a reverse-transcriptase-based mechanism. These platforms may reduce reliance on error-prone double-strand break repair and expand the range of correctable pathogenic variants. High-fidelity Cas enzymes and artificial intelligence-assisted guide RNA design further support improved specificity and efficiency. However, these newer systems also introduce challenges, including editor size, delivery difficulty, editing-window restrictions, bystander edits, variable efficiency across cell types, and incomplete long-term safety data. Thus, next-generation editors should be viewed as major advances, but not complete solutions to the translational barriers facing genome editing (25,26).

Ethical and regulatory issues are especially important because CRISPR/Cas9 affects not only individual patients but also future generations, food systems, ecosystems, and public trust in biotechnology. Somatic editing for serious disease is generally viewed as more ethically acceptable when risks are proportionate and informed consent is robust. Germline editing remains far more controversial because changes may be inherited and because long-term consequences cannot be fully predicted in advance. In agriculture and livestock, ethical questions involve environmental release, biodiversity, food labeling, animal welfare, corporate control of biotechnology, and equitable access for low-resource farming systems. Regulatory frameworks differ widely across regions, creating uncertainty for both clinical translation and agricultural deployment. Responsible implementation therefore requires transparent governance, public engagement, long-term monitoring, and attention to equity as well as technical performance (27,28).

The findings of this review are consistent with the broader literature showing that CRISPR/Cas9 has exceptional scientific versatility but uneven translational readiness. Established evidence supports its use as a research platform, disease-modeling tool, and functional screening method. Strong emerging evidence supports selected ex vivo therapeutic applications and immune-cell engineering strategies.

Agricultural evidence supports broad feasibility for trait improvement, although real-world adoption depends on regulatory and ecological factors. More speculative areas include broad in vivo correction of complex diseases, routine germline intervention, and fully scalable deployment across diverse agricultural systems. Distinguishing these levels of evidence is essential because the speed of CRISPR/Cas9 innovation can encourage overstatement of readiness, particularly when preclinical success is interpreted as clinical or field-level maturity.

This review has several limitations related to its narrative design. The included literature spans heterogeneous disciplines, organisms, disease models, therapeutic strategies, and agricultural systems, which limits direct comparison across studies. No pooled statistical analysis was performed because the evidence base was not organized around a single intervention, population, outcome, or effect measure. Formal risk-of-bias assessment was not applied, and therefore the strength of individual studies was interpreted qualitatively rather than graded quantitatively. The synthesis may also be influenced by publication bias, as successful or innovative CRISPR/Cas9 applications are more likely to be published than unsuccessful or negative findings. In addition, rapidly evolving areas such as prime editing, base editing, clinical trial outcomes, and regulatory policy may change quickly as new evidence emerges.

Despite these limitations, the review provides an integrated perspective on CRISPR/Cas9 across biomedical and agricultural contexts. This broader framing is useful because many of the barriers to implementation are shared across fields. Delivery, specificity, safety, cost, regulation, and ethical governance recur whether the target is a hematopoietic stem cell, a tumor-infiltrating lymphocyte, a crop genome, or a livestock trait. At the same time, the acceptable risk threshold differs substantially across applications. Human therapeutic editing demands the highest safety standards, especially for in vivo or potentially irreversible interventions, while agricultural editing must balance productivity benefits against ecological, food-system, and social considerations.

Future research should prioritize safer and more efficient delivery systems, especially tissue-specific non-viral platforms and transient ribonucleoprotein or mRNA-based approaches. Long-term follow-up studies are needed for therapeutic applications to evaluate durability, late adverse effects, immune responses, and possible oncogenic risks. In oncology, research should focus on improving edited immune-cell persistence, reducing toxicity, overcoming antigen escape, and identifying patient-specific tumor vulnerabilities through integrated genomic screening. In agriculture, future work should emphasize field-level validation, trait stability across environments, biosafety assessment, transparent regulation, and equitable access to genome-editing technologies. Across all domains, interdisciplinary collaboration among molecular biologists, clinicians, agronomists, ethicists, regulators, and public stakeholders will be essential for converting CRISPR/Cas9 from a powerful editing system into a safe, responsible, and broadly beneficial technology.

CONCLUSION

CRISPR/Cas9 represents a highly versatile genome-editing platform with significant potential across genetic medicine, cancer therapy, and agricultural biotechnology. Current evidence supports its strongest translational progress in settings where target cells can be accessed, edited, evaluated, and monitored effectively, particularly ex vivo editing for selected monogenic blood disorders and immune-cell engineering for cancer research and therapy. In agriculture and livestock, CRISPR/Cas9 offers a precise and efficient approach for improving yield, stress tolerance, disease resistance, nutritional quality, and productivity, although broad implementation remains shaped by biosafety, ecological, regulatory, and public-acceptance considerations. Despite its transformative potential, the technology remains limited by off-target editing, delivery barriers, immune responses, repair-pathway constraints, ethical concerns, cost, and unequal access. Future progress will depend on safer delivery systems, improved editing specificity, long-term safety evaluation, transparent governance, and equitable translation so that CRISPR/Cas9 can be applied responsibly in clinical care, precision oncology, and sustainable agriculture.

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