Systemic Review on CRISPR-CAS9 Technology: Unleashing the Power of Precision Genetic Editing

Mr. Abhishek Raosaheb Ghule[1], Mr. Kuldeep Sukhdeo Kanadje[2], Dr. D. K. Vir[3], Mr. Vaibhav Manikrao Hiwale[4], Miss. Pooja Dadarao Gayake[5], Mr. Yash Kishor Pendharkar[6]

[1,2,3,4,5,6]*Pharmaceutical Technology Department, Shree Goraksha College of Pharmacy and Research Center, Chh. Sambhajinagar, India*

Abstract: CRISPR-Cas9 technology has emerged as a transformative tool in genetic editing, offering unparalleled precision and efficiency. Originally discovered as a bacterial defense mechanism against viral infections, this system enables precise targeting and modification of specific DNA sequences. This article aims to examine the potential applications of CRISPR-Cas9 in addressing significant challenges in healthcare, agriculture, and environmental science. The technology relies on two critical components: the Cas9 enzyme, which acts as molecular scissors, and a guide RNA (gRNA) that directs the enzyme to the desired DNA site. By designing tailored gRNAs, scientists can achieve highly accurate gene editing. This method has shown significant promise in correcting genetic disorders, treating cancers, and addressing infectious diseases, as demonstrated by ongoing clinical trials. In agriculture, CRISPR-Cas9 has been utilized to develop crops with enhanced resistance to pests, diseases, and environmental stresses, contributing to improved food security and sustainability.

While the results highlight CRISPR-Cas9's potential, challenges such as unintended off-target effects and the ethical concerns of germline editing, which affects future generations, necessitate careful evaluation. Establishing robust ethical guidelines and fostering public discussions are essential to ensure its responsible application.

In summary, CRISPR-Cas9 is a groundbreaking innovation in genetic editing, offering transformative solutions for global health and agricultural challenges. However, addressing its safety and ethical implications is crucial to realizing its full potential. Continued research and dialogue will play a pivotal role in shaping its future use responsibly.

Keywords: CRISPR–Cas9; Gene Editing; Molecular Genetics; Prime Editing.

1. INTRODUCTION

In 1987, CRISPR was initially found in E. coli and subsequently found in other bacteria. Until 2005, when researchers suggested it as a component of an adaptive immune system against viruses, its function was unknown. CRISPR/Cas9 uses endonucleases to target and cut DNA, allowing for precise genetic changes. CRISPR-based treatments and diagnostics are based on this technology [1]. For genetic engineering, accurate and effective genome alteration is essential. The application of methods that permit modifications to genomes is made possible by the steady advancement of technology. Enzymes, such as zinc finger nucleuses (ZFNs) and transcription activator-like effector nucleuses (TALENs), are the most important tools for genome editing [2]. The distinctive arrangement of brief, partially repeated DNA sequences seen in prokaryotic genomes is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat). Prokaryotes use CRISPR and its related protein (Cas-9) as an adaptive immunity strategy to protect themselves against viruses and bacteriophages [3]. The 2020 Nobel Prize-winning CRISPR/Cas9 gene-editing technique is widely used in clinical and biological research and is very effective at altering genes. However, it still faces challenges, particularly in delivering CRISPR/Cas9 components into the nucleus. Overcoming tissue and cell membrane barriers is essential for effective delivery. Current approaches include physical delivery, viral vectors, and non-viral vectors, with virus-mediated delivery being the most commonly used [4].

CRISPR-Cas9 has facilitated the investigation of gene functions and disease processes in biomedical research. It has enabled scientists to create disease models, perform gene knockouts, and explore potential treatment approaches. For example, CRISPR-Cas9 was used to effectively alter multiple genes at once in a study by Hsu et al., providing a powerful tool for functional genomics research [5].

CRISPR technology provides an effective approach to improving crops by modifying genes associated with yield, disease resistance, and nutritional quality. Demonstrated the application of CRISPR-Cas9 in agriculture by enhancing rice grain yield through the targeted editing of a gene responsible for regulating grain size [6]. The CRISPR/Cas9 system, a genome-editing tool, consists of three key components. The Cas9 endonuclease causes blunt-end double-strand breaks (DSBs) at particular genomic locations under the guidance of single-guide RNA (sgRNA). [7-9]

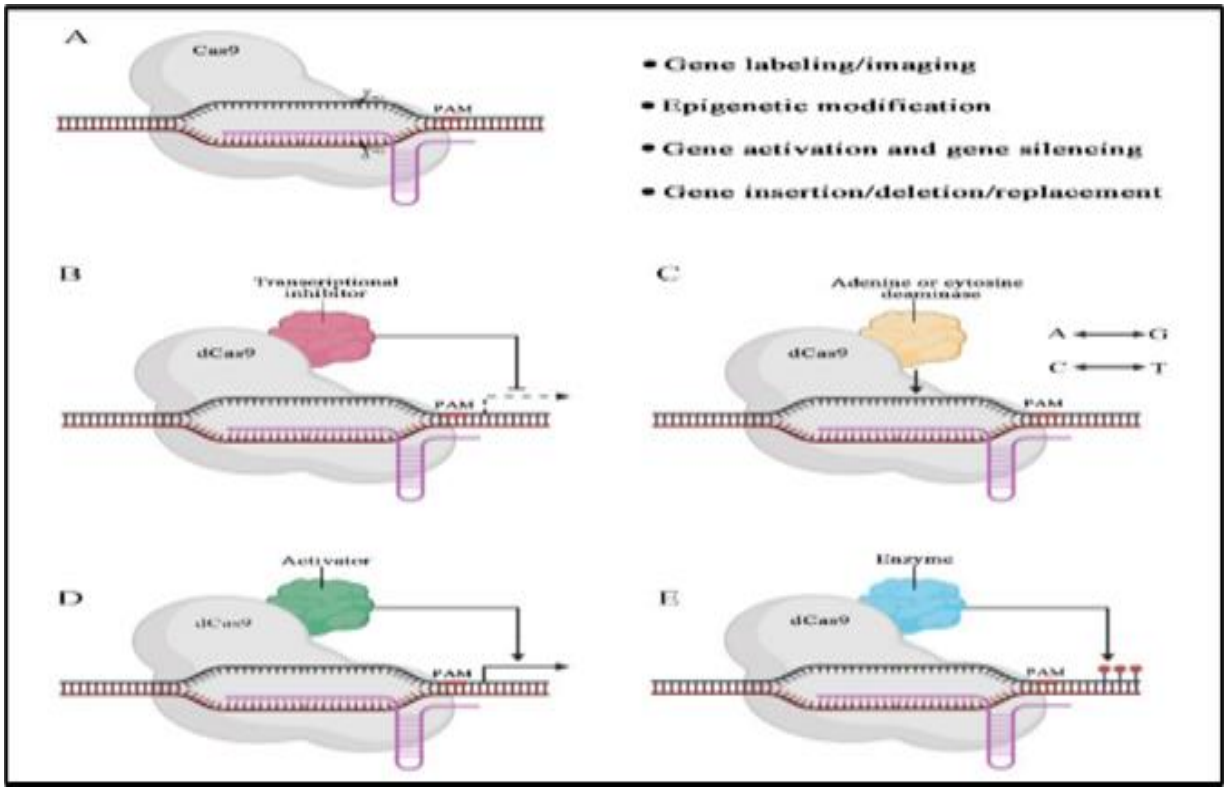

Fig. 1. Applications of CRISPR/Cas9 system. [4]

2. CRISPR ETHICS

Making moral decisions requires evaluating potential profit-loss analyses, particularly in biomedicine. It is preferable to consider the extent of potentially harmful circumstances, the probability of each outcome, and the available justifications for each prospective result when investigating moral dynamics [10]. Sharma and Scot were among the scientists who defended human gene editing under the pretext of "appropriate and justified use." They contend that, in accordance with accepted ethical principles, germ line editing should only be permitted in human embryos that have been cultured for less than 14 days [11-12]. However, Lander strongly supported the ban on technology and was adamantly against the thought of changing human DNA. According to Lander, technology should only be permitted in cases of hereditary diseases for which there is no other viable medical treatment. In addition to being a low-cost, effective, and accurate method for altering a single nucleotide, CRISPR/Cas9 also helps with a variety of scientific and research questions [13]. The development of CRISPR–Cas9 led to significant patent disputes due to its high financial stakes. In 2012, the University of California at Berkeley, the University of Vienna, and Emmanuelle Charpentier filed a patent, closely followed by Feng Zhang and the Broad Institute. While Zhang's patent, focusing on CRISPR in human cells, was granted in 2014, Doudna's was still pending, sparking years of legal battles. By 2019, both parties held patents, with biotech companies licensing from either group. In 2022, the U.S. Patent Office upheld Zhang's claim for CRISPR in human cells, but Doudna's team secured key patents in Europe and several other countries [14].

3. APPLICATIONS OF CRISPR/CAS9

a) Stem Cell Studies: CRISPR/Cas9 technology, enabling gene deletion, knock-in, and activation, has become vital in biological and medical research. It has significantly advanced stem cell studies, essential for tissue repair and regeneration. Approved stem cell therapies show promising clinical outcomes, and combining CRISPR/Cas9 with stem cell research drives significant progress in human health [15].

b) Genetic Disease: Over 10,000 diseases, including 5,000–8,000 monogenic disorders, remain challenging to treat. Key advances include EDIT-101 restoring CEP290 in LCA10, AAV-based exon 23 removal restoring dystrophin, and CRISPR/Cas9 boosting γ-globin for SCD [16].

c) Cancer Therapy: With over nine million annual cancer deaths, understanding the disease remains crucial. CRISPR/Cas9 offers promise for personalized therapies. Clinical trials focus on modified T-cells for immunotherapy, despite challenges like safe delivery and tumor diversity. CRISPR/Cas9 has been used to target EGFR mutations and develop CAR T-cells, achieving remission in relapsed ALL patients [17].

d) Agriculture: CRISPR-Cas9 could revolutionize agriculture by enabling precise genetic improvements in crops and livestock. It enhances traits like disease resistance, productivity, and stress tolerance in crops, while improving meat quality and disease resistance in cattle. Despite its potential to boost production and food security, challenges include GMO acceptance and regulatory issues [18].

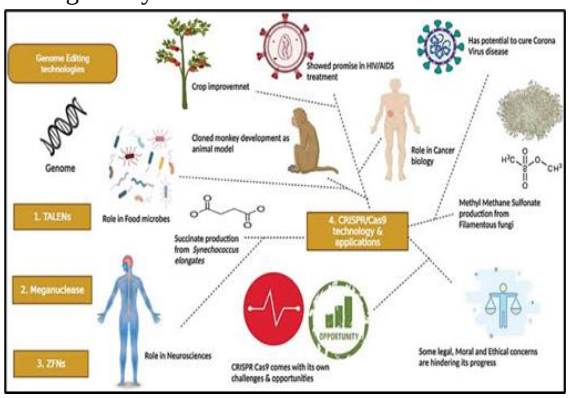

Fig. 2. Application of CRISPR for Cereal Improvement [19]

e) Medicines: CRISPR-Cas9 is revolutionizing medicine by enabling gene function studies, disease modeling, and gene therapy. It supports cancer research, enhances treatments, aids rapid disease detection with tools like SHERLOCK and DETECTR, and corrects genetic disorders like β-thalassemia. Combined with stem cell research, it advances therapy development [18].

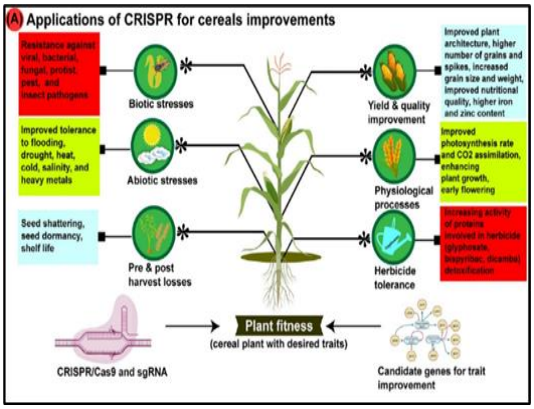

Fig. 3. Applications of CRISPR-Cas9 technology for genome editing in the field of medicine.[20]

f) Cell Lines: CRISPR-Cas9 offers efficient gene modification with high specificity, improving on traditional methods. It enables precise edits for diseases like cystic fibrosis, gene activation/repression using dCas9, and effective genome-wide screening with fewer off-target effects than shRNA [21].

4. CLASSIFICATION

CRISPR-Cas systems consist of two major classes: Class 1 (Types I, III, IV) and Class 2 (Types II, V, VI). Class 2 uses a single multi-domain effector protein, while Class 1 relies on multi-protein complexes. Type II, a Class 2 system, is widely used for gene editing, whereas Types I and III, in Class 1, use multiple Cas proteins and crRNA complexes. The type and subtype of CRISPR-Cas systems are defined by the Cas genes they contain, with key proteins like Cas1, Cas2, Cas3, Cas9, and Cas10 playing roles in immunity and biochemical functions, including nucleic acid recognition [22-23].

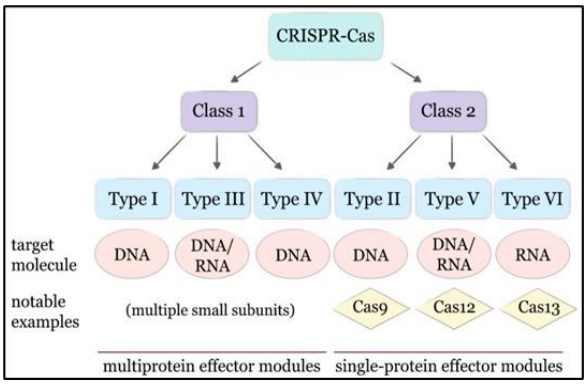

Fig. 4. Conventional classification of known CRISPR–Cas systems. [22-23]

5. MECHANISM OF ACTION

The CRISPR-Cas immune response involves three stages: adaptation, expression, and interference. During adaptation, Cas proteins create double-strand breaks in foreign DNA by recognizing the protospacer adjacent motif (PAM) and integrating the protospacer into the CRISPR array as a new spacer. In expression, Cas proteins process precursor CRISPR RNA (pre-crRNA) into mature crRNA. In interference, crRNA guides Cas proteins to cleave foreign DNA. Type I systems use Cas6e/Cas6f, type II uses tracrRNA and Cas9, and type III employs a Cas6 homolog. In CRISPR-Cas9 editing, the HNH nuclease cuts the target DNA, while the RuvC-like nuclease cleaves the non-target strand. [22]

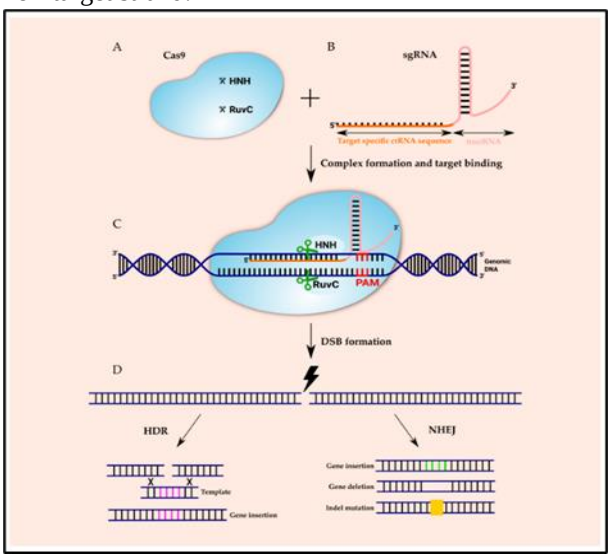

Fig. 5. Molecular mechanism of the CRISPR–Cas9 system. [24]

6. MODERN TARGETED GENOME-EDITING TECHNOLOGIES

Zinc Finger Nucleases (ZFNs), TALENs, and CRISPR-Cas9 allow for targeted genetic modifications. These engineered nucleases induce double-strand breaks (DSBs) at specific locations in the genome, which are then repaired through either homology-directed repair (HDR) or non-homologous end joining (NHEJ) [25-26].

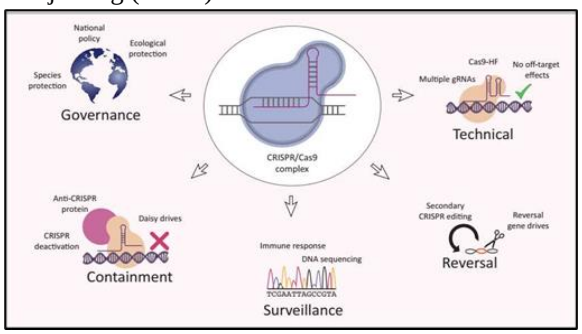

Fig. 6. Promoting safety in genome editing.[26]

6.1. ZFN's

Zinc finger proteins (ZFPs) are DNA-binding transcription factors used as endonucleases for gene editing. The FokI-derived cleavage domain, surrounded by 4–6 ZFPs, targets 18 base pairs. ZFPs, typically 30 amino acids in length, utilize homology-directed repair (HDR) or non-homologous end joining (NHEJ) for gene editing in both prokaryotes and eukaryotes. Since their discovery in 1996, ZFNs have been used to modify genes in plants like Arabidopsis and maize, offering high target specificity and limited off-target effects [27-28].

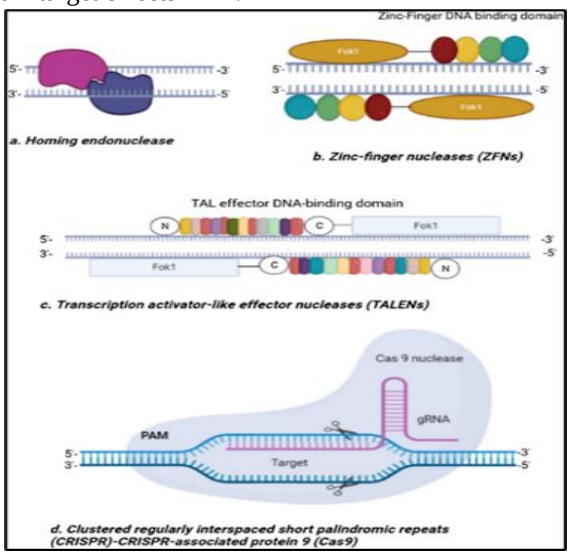

Fig. 7. General assembly of major genome-editing technologies.[20]

6.2. TALEN's

In the same way as ZFNs do, TALENs have two domains: one for the target site of DNA cleavage and another for DNA binding. This approach is a potent tool for gene editing in several types of living things, including rats, zebra fish, chicken, frogs, certain cells belonging to mammals, the tomato plant, tomato, wheat, rice, and potatoes, for this domain system [28-30].

7. HISTORICAL OVERVIEW OF CRISP-CAS9-MEDIATED GENOME EDITING

CRISPR-associated (cas) genes, located near CRISPR loci in many prokaryotes, contain helicase and nuclease-like motifs, suggesting their role in DNA cleavage. Their connection to CRISPR arrays, identified by Eugene Koonin's team in 2002, was initially not understood [31].

A diverse array of CRISPR-associated genes has been identified in prokaryotes, including helicase, nuclease, and polymerase families. These proteins are classified into the adaptive module, which facilitates immunity acquisition (e.g., Cas1 and Cas2), and the effector module, which targets and cleaves mobile genetic elements. Additionally, some regulatory proteins are associated with the system [32].

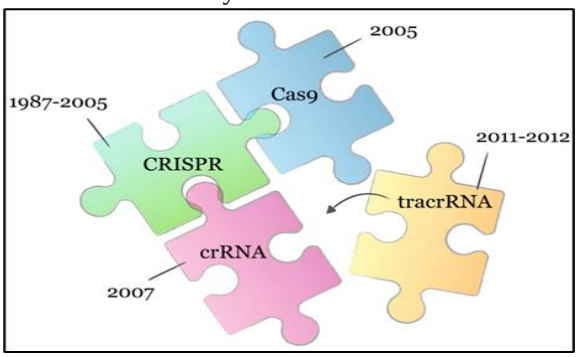

Fig. 8. Historical timeline of discoveries of the components of the CRISPR–Cas9 system.[33]

CRISPR-Cas systems, comprising two classes and six types, include the widely studied Cas9 nuclease, known as "genetic scissors." Initially called Cas5 by Bolotin et al., it shares an HNH motif with other nucleases. They also identified protospacer adjacent motifs (PAMs) near CRISPR spacers, crucial for CRISPR activity [34-35].

The prokaryotic immune system targets DNA fragments matching CRISPR spacers but requires a PAM pattern for recognition. The CRISPR-Cas9 system uses crRNA, processed from pre-crRNA, to direct immune proteins to foreign genetic material [36]. Virginijus Siksnys' team demonstrated that while CRISPR spacers are longer, the CRISPR-Cas complex's nuclease activity requires only a 20-base-pair crRNA sequence complementary to the target DNA [37].

8. PRE-CLINICAL STUDIES OF CRISPR-CAS9 FOR DIFFERENT DISEASES

CRISPR-Cas9 research has been applied for the treatment of different human diseases, which are discussed below.

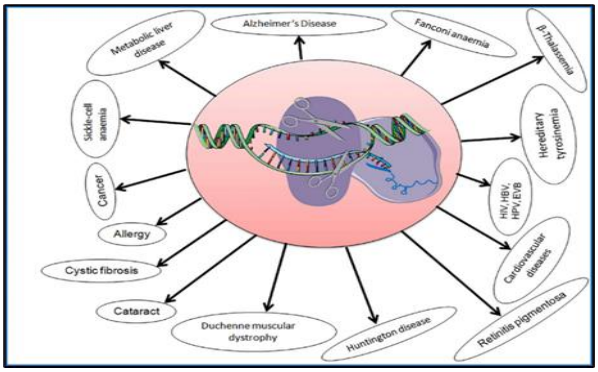

Fig. 9. CRISPR-Cas9 System Dealing for Treatment of Multiple Human Diseases [38]

8.1. Allergy and Immunological Disorders

CRISPR-Cas9 technology is being explored for treating allergic and immune conditions. Here are some key findings:

- Chronic Obstructive Pulmonary Disease (COPD) and Asthma
- Immune Response Enhancement
- X-Linked Hyper IgM Syndrome
- JAK3 and Allergic Asthma [38]

8.2. Cardiovascular Disorders (CVDs)

The PCSK9 gene regulates cholesterol levels, and its mutations can lead to hypercholesterolemia and atherosclerosis. Researchers are using CRISPR-Cas9 to target this gene for treatment.

- Mouse Studies
- Zebrafish Research [38]

8.3. Neurological Disorders

CRISPR-Cas9 technology can be used to target hereditary neurological illnesses such as Alzheimer's disease (AD) and Huntington's disease (HD).

- Huntington's Disease (HD)
- Alzheimer's Disease (AD)
- APP Gene Mutations [38]

9. CLINICAL TRIALS

Targeting the PD-1 gene in T cells, China carried out the first ex vivo clinical trial with CRISPR-Cas9 for patients with metastatic non-small-cell lung cancer. Although the researchers were able to identify the altered T cells in the patients' blood, they stressed that better gene-editing tools are required to increase the efficacy of treatment. Stadt Mauer et al. introduced a transgene that detects malignancies while editing T cells to eliminate genes encoding the PD-1 locus (PDCD1) and T cell receptors (TRAC and TRBC). Despite some chromosomal translocations, the therapy was well tolerated for up to nine months.

By incorporating two CARs into T cells to identify CD19-cells, a clinical study for CAR T cell treatment targeted relapsed hematological malignancies. Furthermore, a study employing allogeneic universal CD19-specific CAR T cells employed electroporation to interfere with genes that might result in GVHD.

A study (CTX130) that targets CD70 in hematologic malignancies was approved by the FDA. The first in vivo gene-editing therapy was used in a novel trial to treat Leber's congenital amaurosis 10 (LCA10) by injecting a CRISPR-Cas9 medication straight into the eye. CRISPR-Cas9 technology is currently being used in roughly 19 registered clinical trials for a variety of diseases [39].

10. DISCUSSION

CRISPR-Cas9 is a groundbreaking genetic editing tool that allows for precise modifications to DNA in a variety of organisms. Derived from a natural defense mechanism in bacteria, CRISPR-Cas9 utilizes a guide RNA to target specific sequences in the genome, while the Cas9 enzyme acts as a molecular "scissors" to cut the DNA. This technology has revolutionized genetic research, enabling scientists to knock out genes, insert new genetic material, or even edit genes at specific locations with unprecedented accuracy. In medicine, it holds promise for treating genetic disorders, enhancing cancer therapies, and developing personalized medicine approaches. In agriculture, it can create crops that are more resilient to disease and environmental stress. Furthermore, CRISPR's role in biotechnology extends to advancements in biofuels and pharmaceuticals. However, the technology is not without ethical and safety concerns. Issues such as off-target effects, ecological impacts, and the moral implications of editing the human germline raise important questions that must be addressed as CRISPR applications move forward.

11. CONCLUSION

CRISPR-Cas9 technology represents a significant leap forward in our ability to edit genomes with precision and efficiency. Its potential to revolutionize medicine, agriculture, and various scientific fields is immense, offering solutions to some of the world's most pressing challenges, such as genetic disorders, food security, and sustainable energy production. The capacity to modify genes quickly and accurately could lead to groundbreaking therapies for conditions that were once deemed untreatable, enhance crop resilience in the face of climate change, and drive innovations in biotechnology. However, the rapid advancement of this technology necessitates a careful consideration of ethical, regulatory, and safety aspects. The potential for unintended consequences, such as off-target effects or ecological disruptions, underscores the importance of robust research protocols and comprehensive risk assessments. As we continue to explore the possibilities of CRISPR-Cas9, fostering an inclusive and informed discourse will be essential to ensure responsible use and governance of this powerful tool. A collaborative approach can help navigate the complexities of genetic editing, balancing innovation with caution. The future of genetic editing is bright, and with it comes the responsibility to harness its power wisely, ensuring that its benefits are shared equitably across society while minimizing potential risks.

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