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Resolving Gene Editing Technology's Ethical and Regulatory Challenges

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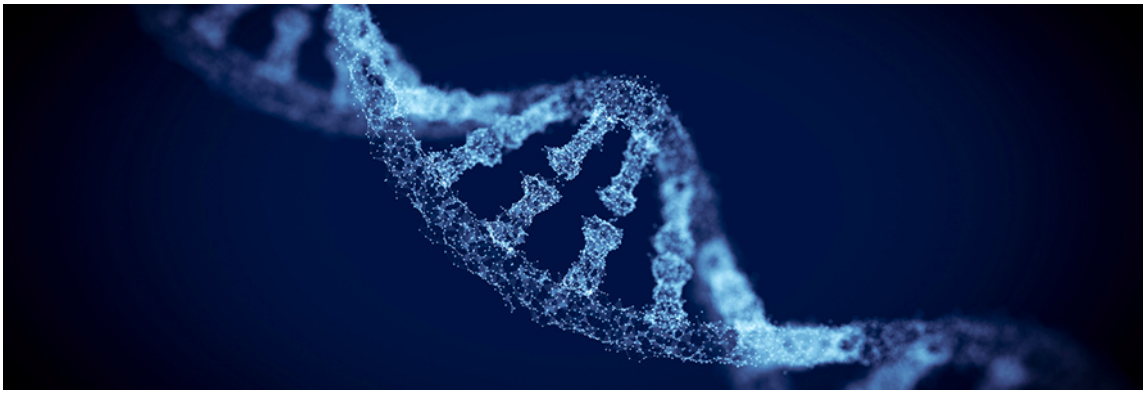


This article provides an overview of biomedical applications of gene editing technology, addresses ethical and regulatory challenges associated with its implementation for therapeutic development, and proposes approaches for overcoming these challenges.

Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology is a genetic "editing" tool aimed at altering DNA sequences and modifying gene function. It offers promising

opportunities for ameliorating genetically based diseases beyond the reach of conventional therapies. However, CRISPR technology also raises ethical and regulatory concerns that must be resolved. Resolving these issues means establishing streamlined regulatory policies to address ethical implications and ensure the long-term safety and efficacy of therapeutics derived from this gene editing technology.

Rapid developments in genetics over the past few decades have revolutionized the field of biomedical sciences and have enabled advancement in the prediction, diagnosis and treatment of many diseases. Better knowledge of the genetic basis of diseases had led to the use of "gene therapy" which allows for a disease-causing gene to be replaced with a healthy copy of the gene, reversing the disease.[1] Recent advances in gene editing technology have allowed manipulation of the eukaryotic genome by using target-sequence-specific engineered nucleases allowing both precise correction of disease causing mutations, the addition of therapeutic genes to specific sites in the genome, and the removal of deleterious genes.[2] "Gene editing" technology is now being aggressively pursued as a "next-generation" therapeutic approach to treat a wide range of diseases at the genetic level. These genetic diseases include hereditary, infectious, neoplastic, and neurodegenerative diseases. [3,4]

Gene Editing Tools

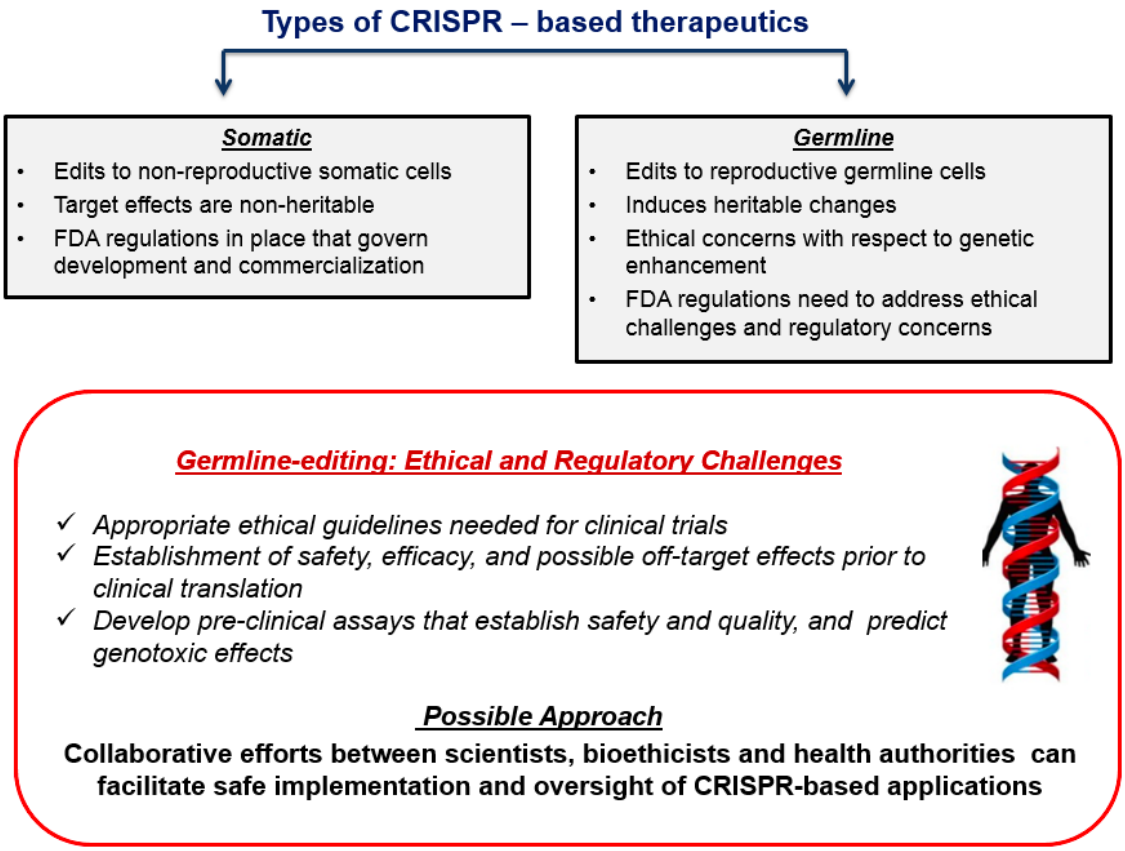
Gene editing tools include nucleases that are restriction enzymes with engineered DNA-binding domains which produce

double-stranded breaks.[5] Recent discoveries include Zinc Finger Nuclease (ZFN) and Transcription Activator-Like Effector Nuclease (TALEN). TALEN, a technique garnering attention in the world of gene editing, is a Clustered Regularly Interspaced Short Palindromic Repeat or CRISPR technology.[6,7] The CRISPR system was first discovered as a component of immune system in bacteria.[8] With this technology, the nuclease protein Cas9 is targeted to a genomic site in cells by a segment of guide RNA and generates a double-strand break in DNA, followed by DNA repair processes in the presence of a donor template.[9] CRISPR replaces other gene editing tools because it is inexpensive and involves simplified processes, from design to execution, that facilitate gene target identification, validation and therapeutic development.[10,11]

CRISPR Applications

CRISPR technology applications are several and growing quickly (**Figure 1**). For example, targeted induction of genetic changes using CRISPR has allowed creation of disease model systems for discovery research and enabled bio-engineers to generate healthy and resilient plant crops and livestock.[12] CRISPR applications extend to altering the ecosystem through a method called "gene drive," allowing a genetic mutation to be rapidly propagated through generations. CRISPR/Cas9 editing also has been used to target malaria mosquito embryos, offering a promising approach for malaria eradication by either impairing the fertility of female mosquitos or inserting an antimalarial gene into malaria mosquitos.[13]

Figure 1. Types of CRISPR-Based Therapeutics



For biomedical applications, permanent gene modification in living cells and organisms will aid in the development of novel treatment approaches for diseases with a genetic basis, including cancer, diabetes, and Alzheimer's disease. CRISPR also provides hope for developing a novel, powerful class of gene therapies that allow for the addition, deletion, or repair of flawed, disease-causing genes. For example, CRISPR has been successfully used in mice to correct a mutation associated with tyrosinaemia, a human metabolic disease.[14] Also, a recent study demonstrated successful editing of post-mitotic neurons in the adult mouse brain following injection of Cas9 ribonucleoprotein complexes in the hippocampus, striatum and

cortex, indicating the potential for CRISPR to correct the underlying genetic causes of neurological diseases.[15] CRISPR also was successfully employed in developing a diagnostic screening tool for Parkinson's disease when scientists were able to 'light up' and monitor alpha-synuclein, a brain protein associated with Parkinson's disease.[16] Measuring this "reporter protein" allows for investigations of disease progression and facilitates high-throughput screening of drugs by determining alteration of alpha-synuclein levels in patients.

Additionally, CRISPR technology can assist with disease modelling, facilitate understanding of potential underpinnings of the initiation and development of human diseases, and expedite discovery of novel therapeutics. While "channelling" this technology in the right direction can lead to transformative discoveries, significant fundamental and translational work demonstrating safety and specificity, and work that establishes appropriate delivery strategies, is needed to optimize the therapeutic potential of CRISPR and make CRISPR/Cas9-based therapies a reality.

Ethical and Regulatory Issues

When successfully applied at the preclinical level, genome editing using CRISPR suggests that the technology offers promising therapeutic potential for diseases with heretofore unmet medical needs.[17] For example, in China, the first country to test this revolutionary gene editing technology on humans for cancer intervention, researchers extracted immune cells from a lung cancer patient and used CRISPR/Cas9 to "disable" the

gene expressing the protein PD-1, thereby preventing cancer cell proliferation. The edited cells were cultured and injected back into the patient in hopes of combating cancer.[18] Despite the promising therapeutic potential of CRISPR gleaned from preclinical studies, ethical and regulatory concerns in the US are preventing clinical trials using the technology.

Ethical Concerns

The therapeutic objective of human genome editing is to treat, ameliorate or prevent eventual disease phenotype presentation. Theoretically, CRISPR can eliminate root causes of hereditary human diseases through correction of the defect in reproductive cells. Edits to the human genome can either be to somatic (non-reproductive) cells or to heritable germline cells. When germ-line genomic edits are made, they may be focused on enhancement of intelligence or physical traits.[19]

Data compiled from the International Summit on human gene editing suggests that a majority of Americans approve of gene editing approaches (both somatic and germline edits) for treating patients with serious, genetically-based diseases or for preventing offspring from inheriting potentially fatal genetic diseases.[20] Although a majority of Americans support gene editing for improving health, a majority do not support modification of the human genome through germ-line or embryonic alterations, especially for potentially eugenic purposes. Therefore, like any other gene editing technique, ethical implications of CRISPR are directly related to the purposes for which they are used.[21]

In addition to offering therapeutic solutions, CRISPR editing of germ cells also could facilitate the creation of "designer babies" with superior, desirable genetic characteristics.[22] This possibility has raised ethical concerns. Policy debate over the past several decades has strongly concluded that such a use would be ethically unacceptable as the techniques could, many argue, propagate injustice, crime and create a divide in the society. CRISPR/Cas9-based genome editing in an embryo is also dangerous and could have unpredictable and undesirable consequences on future generations as it is difficult for researchers to predict the effects of such procedures before birth as quality control can be performed only on a subset of cells.[23] Therefore, it may be impossible to know with precision the effect of genetic modification of an embryo until after a birth as consequences cannot be investigated or identified at the pre-natal stage.

Clinical research involves "informed consent" whereby investigators educate prospective research participants regarding potential risks and benefits associated with the clinical study. In the US, this occurs through oversight by an Institutional Review Board (IRB), as per regulations established by the Food and Drug Administration (FDA) and the Department of Health and Human Services. The off-target effects and gap in knowledge with respect to safety and long-term side effects associated with CRISPR complicates the informed consent process. Similarly, the IRB process for conducting clinical trials poses challenges to healthcare practitioners with respect to educating the patients as it is not clear exactly what information

would be needed from or provided to prospective parents to adequately inform them about the risks involved in germline modification. Unfortunately, ethical concerns surrounding genome editing of the human embryo using CRISPR are hindering gene editing of somatic cells that present a promising area of therapeutic development. Human medical products that apply gene editing to exert their therapeutic effect are regulated under FDA's existing framework for biological products. The framework regarding "gene editing" refers to non-heritable situations, such as somatic cell gene therapy, and not to heritable conditions, such as germ line gene therapy. Because of this, it is necessary to establish a clear distinction between somatic and germline interventions and establish appropriate ethical guidelines for clinical trials.

Regulatory Concerns

From a regulatory perspective, safety is the most important issue with regard to gene editing and pertains to the possibility of genotoxicity through the modification of non-target genes.[24,25] Genetic modifications are permanent and deleterious off-target mutations could create cells with cancer-causing potential, potentially leading to functional impairment and devastating consequences. For that reason, it is imperative to conduct preclinical safety studies to define and detect off-target effects and devise strategies to minimize or eradicate them prior to clinical development. The major challenge here is to establish preclinical assays for assessment of genotoxicity that can be validated to predict potential genotoxic risk.

Another issue facing clinical translation of therapeutics emerging from CRISPR is optimum delivery to target cell types. Given that nucleases may exhibit off-target cleavage activity or trigger immune responses, the delivery system should be carefully selected and optimized for safety and efficacy.[26] Another drawback comes with the assessment of quality of cells resulting from germline intervention. Quality control can only be conducted on a subset of cells, with the precise effects of genetic modification to an embryo known only after birth.[27]

FDA's "expanded access pathway" allows using an investigational medical product outside of a clinical trial for the diagnosis, monitoring or treatment of a serious disease or condition.[28] The promising therapeutic potential of investigational therapeutics derived from CRISPR is likely to stimulate demand from patient groups, especially those suffering from serious conditions lacking treatment options. It is important to establish regulatory requirements governing expanded access of CRISPR therapeutics to patients in dire need of treatment options. In the absence of adequate regulatory and ethical oversight, it is possible that somatic or germline interventions may be offered to patients ahead of sufficient testing aimed at confirming safety and efficacy. Of course, as with any other gene editing technology, misuse of CRISPR could have potential catastrophic consequences and ease of access and execution, as well as flexibility of CRISPR, may lead to misapplication through bioterrorism activities. For these reasons, regulations should restrict the use of this technology to only highly trained and responsible professionals.

Finally, given the breadth of applications associated with CRISPR, it is imperative to establish a streamlined regulatory framework that addresses the ethical implications and ensures long-term safety and efficacy of products emerging from this technology.

Solutions

Although we are still grappling with policy concerns regarding gene editing approaches for treating genetically based clinical disorders, significant progress has been made with regard to delivering potential solutions to treat, and even eliminate, genetic disorders. While ethical issues are unresolved, especially with regard to alteration of germ-line or embryonic structural genomic material, there may never be consensus on a complete set of clinical circumstances in which a CRISPR/Cas9 based-treatment can be utilized in a clinical setting. However, a starting point for discussion leading to that reality could include the following:

- Delineation of the circumstances under which this technology can/cannot be used. To successfully leverage the therapeutic potential of CRISPR/Cas9 technology, an efficient and streamlined regulatory framework is necessary to facilitate successful transition of therapeutics from clinic to market. This would ensure that the safety and efficacy of such approaches are thoroughly evaluated through both *in vitro* and *in vivo* models to minimize the risks to clinical populations. To avoid potential threats to public health, stringent regulations and protocols should be enforced restricting use of this powerful

enabling tool by qualified professionals.

- To solve ethical dilemmas, solid regulations are essential to govern safety and ethical concerns during conduct of clinical trials.
- It is also essential to demonstrate safe outcomes and obtain reproducible data over multiple generations.
- Promotion of collaborative efforts between scientists, bioethicists, legal experts, and health authorities to establish ethical boundaries, devise strict protocols and regulations that facilitate safe implementation and oversight of CRISPR-based applications at a global level.
- Full compliance with guidelines established through projects including the 'Safe Genes' initiative created by the US Defense Advanced Research Projects Agency. These types of projects promote research for improving accuracy and safety of CRISPR/Cas9 and other gene modification approaches in clinical applications.

Conclusion

The development of new scientific and therapeutic approaches for use in clinical applications to address human genetic diseases has the potential to significantly impact patient outcomes in a wide variety of disorders, including oncology, the rare disease space, and neurodegenerative conditions. CRISPR/Cas9 technology has the potential to address genetically-based heritable disorders through modification of gene sequences in their "native environment" at the nucleic acid

level and within chromatin itself. However, these scientific and technological advances cannot achieve their potential to improve the human condition without full consideration of associated and inherent regulatory and ethical implications. A comprehensive understanding of the technology, including potential error rates, long-term outcomes, associated morbidities, and other variables, must be achieved. Moreover, the specific circumstances under which such powerful and possibly permanent species-altering approaches are utilized clinically must be thoroughly evaluated.

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