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Balancing Progress and Ethics: Exploring the Science and Ethics of Gene Editing: Literature Review

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Balancing Progress and Ethics: Exploring the Science and Ethics of Gene Editing Literature Review

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Introduction

Two decades ago, the completion of the Human Genome Project marked a pivotal milestone in scientific history, unraveling the blueprint of human DNA and laying the foundation for the Genetic Revolution. This monumental achievement allows researchers to uncover the correlations between DNA sequences and biological functions. Today, this movement has propelled modern geneticists toward the peak of human health sciences: the ability to manipulate the very essence of our being. With the aid of Genetic Engineering, scientists now possess the capability to alter the fundamental framework of our genetic code, offering the potential to cure fatal genetic disorders alongside other new therapeutic applications. With this powerful application geneticists have hit a break: determining the ethical boundaries that govern the utilization of this powerful technology. The line between therapeutic interventions and ethically uncertain practices, such as human enhancement or reckless experimentation, grows increasingly tenuous. Despite the remarkable promise of gene editing, it is necessary to navigate this ethical predicament cautiously. Gene editing holds immense promise as a therapeutic tool, continually making groundbreaking discoveries. However, this progress is accompanied by ethical and technological problems that require consideration. To present the current landscape of gene editing, this literature review focuses on the latest advancements in genetic engineering, examining its applications, ethical dilemmas, and the regulatory debate in the United States. This review aims to provide insights into the rapidly changing dimension of gene editing, understanding its potential, challenges, and governance in biomedical research.

Background

What is Human Gene Editing?

Human gene editing involves precisely modifying specific DNA sequences within the human genome through techniques such as deletion, insertion, or modification. DNA, or deoxyribonucleic acid, constitutes a molecular chain carrying an organism's unique genetic information, governing various biological processes including embryonic development and organism functions. Genes, designated sections of DNA, encode specific molecular functions. Modern gene editing gives scientists the ability to alter the DNA sequences of particular genes, thereby modifying their functions. Unlike older methods that involved random insertion of genetic material into the human genome, current gene editing techniques enable precise targeting of specific genes, giving more efficient and faster outcomes (Chen & Chen, 2019, pp. 1-2). Depending on the type of edit, whether it be deletion or insertion, the resulting genetic alterations can vary. Deleting segments of gene code can disrupt regulatory elements, potentially increasing gene expression by eliminating inhibitory factors. Alternatively, cutting specific nucleotide segments (ACGT) within genes can either enhance or inhibit gene function altogether (Chen & Chen, 2019, pp. 1-2). Genes encode the structure and function of proteins crucial for cellular processes. Altering nucleotides within genes can ultimately affect protein expression, structure, and function, with implications that may prove beneficial or detrimental depending on the therapeutic situation (Lanigan et al., 2020, pp. 1-4). Gene editing typically involves two complementary components: a protein structure facilitating edits and a vector guiding the protein to the targeted DNA segment. However, off-target edits may arise, potentially complicating or worsening the condition during therapeutic applications (Lanigan et al., 2020). The Human Genome Project and modern genetic research now has the capacity to identify correlations

between specific genes and their functions. With this knowledge, clinical geneticists can develop patient-specific therapies tailored to individual genetic codes, aiding in the treatment of genetic disorders. Successful gene editing tools must exhibit minimal off-target effects, cost efficiency, ease of production, proficiency in breaking DNA double-stranded breaks, and high selectivity (Carroll, 2017, pp. 2). Combining the safety, costs, and molecular application determines the preferred editing tools for both research purposes and potential therapeutic applications.

Current Gene Editing Methods

TALENs

A pathogenic bacterium called Xanthomonas utilizes Transcription Activator-Like Effector (TALE) proteins, secreted to infect various plant species. These TALE proteins grant the bacteria the capability to induce virulence within the nucleus of the infected plant cells. The protein acts as a transcriptional activator, and facilitates the transcription of DNA into mRNA, subsequently coding for a new DNA sequence. By combining a specific TALE protein with a non-specific endonuclease, Fok I, capable of recognizing and cleaving specific sections of a gene, researchers can create TALEN (Transcription Activator-Like Effector Nucleases), a tailored restriction enzyme (Chen & Chen, 2019, pp. 3). Specific segments of amino acids, referred to as Repeat Variable Diresidues (RVDs), within the TALE protein possess the ability to recognize specific DNA nucleotides with varying degrees of success, enabling precise targeting of a specific gene. When two of these TALEN proteins are present, the selected gene can be approached from both sides. Through the processes of transcription and translation, the targeted gene can be modified (Chen & Chen, 2019, pp. 3). This gene-editing tool provides geneticists with another way to edit the genome for both therapeutic application and research. If scientists

continue to use this technology, they may be able to enhance it as gene editing science progresses. Talens are deemed more cost-effective and specific compared to Zinc Finger Nucleases (ZFNs), yet they still carry the potential for undesired mutations and alterations (Yeadon, 2014, para. 20). In conclusion, TALENs offer a promising tool for precise gene editing, though ongoing research is essential to optimize their efficacy and safety. As geneticists continue to use this tool, the development of new techniques and gene editing apparatuses may be done more effectively.

ZFNs

Zinc Finger Nucleases (ZFNs) are composed of a fused zinc finger-specific DNA agent combined with a non-specific DNA cleaving agent. This combination creates an artificial restriction enzyme typically comprising 3-6 zinc finger segments, each capable of recognizing a corresponding number of base pairs, similar to TALENs. The primary cleaving agent utilized in this gene editing technique is Fok I, similar to its usage in TALENs (Chen & Chen, 2019, pp. 3). ZFNs operate similarly to TALENs, with two enzyme complexes approaching the DNA segment from both sides. The engineered zinc finger segments have specificity for certain nucleotides, enabling the identification and alteration of target gene segments. DNA regions rich in cytosine amino acids often pose a challenge for this form of gene editing (Chen & Chen, 2019, pp. 3). Zinc Finger Nucleases were one of the first endonuclease genome editing tools and were considered relatively primitive compared to more modern techniques. Due to the presence of zinc markers throughout the genome, ZFNs can inadvertently affect neighboring genes, potentially leading to issues such as mosaicism or undesired mutagenesis. Among the three major gene editing tools, ZFNs are considered the least cost-effective and least specific (Yeadon,

2014). While ZFNs may find utility in model organisms and hold potential for refinement, they are unlikely to be the preferred tool for therapeutic genetic engineering.

CRISPR-Cas9

The CRISPR-Cas9 system originated from bacteria as an adaptive defense mechanism against foreign DNA, such as plasmids or viral DNA. This system has emerged as the most promising, cost-efficient, and safest approach to gene editing, which ultimately means its the primary technique focused in the literature review. CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, refers to specific DNA loci containing short repeated sequences of nucleic base sequences. Each repeat is linked to a segment of spacer DNA, composed of sequences foreign to the organism. The key protein associated with this complex is Cas9, functioning as a DNA endonuclease similar to Fok I. Cas9 comprises two lobes, one for target recognition and the other as a multi-nuclease structure catalyzing DNA cleavage. Nucleases within Cas9, such as RuvC, His-Me (HNM), and a carboxyl-terminal domain, recognize the protospacer adjacent motif (PAM), a short DNA sequence following the targeted DNA segment, essential for CRISPR system cleavage. The CRISPR system includes CRISPR RNAs (crRNAs), Trans Activating crRNAs, Precursors crRNAs, and Cas9 endonuclease. Trans Activating and Precursors crRNAs form a duplex guiding Cas9 to foreign DNA for cleavage. RNase III cleaves these components to form single guide RNA, allowing accurate genome cutting. The only limitation is the presence of the PAM sequence on the target gene is necessary in order for the system to work (Chen & Chen, 2019, 3). While CRISPR was initially discovered in the late 1980s as a bacterial defense mechanism, its combination with Cas9 and practical application were introduced by Emmanuelle Charpentier and Jennifer Doudna. Recognized for their

groundbreaking work, they were awarded the Nobel Prize in Chemistry in 2020 (Gostimskyata, 2022, 4). CRISPR-Cas9 stands as the most efficient genome editing tool, holding immense potential for clinical applications. Ongoing advancements continue to slow CRISPR's limitations and molecular malfunctions, allowing its widespread usage in gene editing and therapeutic applications. These applications include but are not limited to altering gene expression in aggressive cancers or altering function for genetic disorders. Applications are discussed in more depth in the clinical applications portion of this literature review.

Germline Vs Somatic/Therapeutic Edits

When discussing genetic engineering, it is important to understand the classifications of the different types of gene therapies as it determines the type of cells being edited, which could lead to different cascades of events and influence the regulation of the therapy. Germline gene editing involves using a genetic engineering tool to create a heritable in vitro edit in an early-stage embryo gene, precursor germ cells, or gametes such as eggs or sperm. These edits lead to the development of the embryo, adhering to those new edits, and resulting in offspring born with those edits. These edits are heritable, meaning the edited offspring would pass these traits down to their offspring with their edited gamete cells (Baylis et al., 2020). Therapeutic gene editing, often considered somatic gene editing, involves using a genetic engineering tool to edit a non-reproductive cell's gene, known as a somatic cell, which has little chance of being passed down to further generations. These edits typically occur outside of the body with the specific gene editing tool and are then reintroduced into the body (NIH, 2024). The major highlighted difference between the two types of editing is whether the edit is heritable or not, which depends on the cell being edited (gamete vs. somatic cells).

Literature Review: Beneficial Clinical Applications and Future Projections

CRISPR Mediated Cancer Treatments

A major portion of medical-based scientific research, especially in genetics, revolves around the prevention and treatment of cancer. According to the National Cancer Institute (2020), mutations in the human genome lead to rapid, uncontrolled cell division, commonly known as cancer. Geneticists and cancer scientists have concluded that cancer can be prevented or detected early with genetic tools such as gene mapping and editing. However, before the current decade, many of these tools were costly and lacked operational potential compared to modern technologies (NCI, 2020). With the introduction of CRISPR technology, cancer scientists became propitious about its potential in treating and preventing cancer caused by genetic mutations. The first clinical trial for cancer treatments using CRISPR was conducted in 2019 at the University of Pennsylvania. This trial involved modifying T-cells which are important immune cells for defending against infection, to enhance their ability to identify and eliminate specific cancer cells which in this case are NY-ESO-1 receptors on sarcoma and myeloma cells. The trial concluded that CRISPR T-cell modification is safe with minimal side effects and off-target errors. However, it only resulted in a slowing of cancer growth, with an overall effectiveness of about 66% in the clinical group (NCI, 2020). The findings suggested that while CRISPR holds promise for cancer treatment, further research is necessary to fully comprehend its long-term effects. Nonetheless, CRISPR has the potential to be highly effective in treating cancers through various avenues (NCI, 2020). In summary, CRISPR technology presents promising avenues for cancer treatment, though further research is needed to fully understand its long-term effects and optimize its accuracy. Working to understand its role at the molecular level can help geneticists further increase its effectiveness.

Recently a new concept termed "cancer shredding" has emerged in the field of gene editing biotechnology, created by researchers at the University of California and the Gladstone Institute of Data Science and Biotechnology. This innovative technique involves cancer-specific CRISPR treatments targeting hyperactive and hypermutated cancers. Primary glioblastoma, a particularly challenging hyperactive cancer of the central nervous system, poses significant treatment obstacles as surgical treatment is often impractical due to the invasive nature of targeting brain tumors. Through genetic cell sequencing, scientists have identified the heterogeneity of this cancer, revealing high genetic mutation rates that disrupt normal genetic processing. While inhibiting these cellular processes to halt tumor growth may appear feasible, the tumor's heterogeneity allows growth to persist from various mutated clones. CRISPR technology presents a promising solution by targeting the mutated genes responsible for cloning and tumor growth, offering a safer and less invasive treatment approach for glioblastoma. In addition to surgical challenges, glioblastoma treatment typically involves chemotherapy with temozolomide (TMZ), which extends patient survival but fails to halt cancer progression and may even worsen it due to the sensitivity of the O-6-methylguanine-DNA methyltransferase (MGMT) promoter in each patient. The level of MGMT inhibition varies among each case, with higher inhibition correlating with increased susceptibility to DNA damage from TMZ and subsequent hyperactivity of the cancer (Tan et al., 2023, pp. 1-24). It is clear that the new approach of "cancer shredding" addresses the challenges posed by glioblastoma, particularly its heterogeneity and resistance to conventional treatments like chemotherapy with temozolomide (TMZ). This targeted CRISPR therapy offers the potential for a safer and more effective treatment option, especially for aggressive cancers.

In this experiment, researchers are utilizing CRISPR technology to target and cut specific clones in the genome responsible for tumor growth (cancer shredding). This innovative treatment approach aims to eradicate cancer cells derived from recurrent glioblastoma patients while sparing normal cells, presenting a promising strategy for addressing hypermutated cancers regardless of their genetic and epigenetic characteristics. By leveraging the tumor mutational burden (TMB) and the mutational signature induced by temozolomide (TMZ) as potential therapeutic targets, this approach holds significant promise for effective therapy. The study revealed a large number of mutations in the non-coding genome, which is often overlooked in targeted therapies along with underlining the limitations of targeting the mechanism responsible for tumor growth. Identified germline mutations in DNA damage response genes and somatic mutations crucial for glioblastoma cell growth further underscored the complexity of the disease. CRISPR targeting of highly repetitive sequences in the genome, known as "sgCIDEs," showed potential in selectively eliminating glioblastoma cells across various genetic backgrounds and variations. This approach induced rapid cell death independent of TMZ sensitivity or MGMT promoter sensitivity status. Moreover, both in vitro and in vivo experiments demonstrated the effectiveness of genome shredding in destroying glioblastoma cells and overcoming treatment resistance. This research represents a significant advancement in the field of cancer treatment, offering a promising therapeutic approach for addressing hypermutated cancers like glioblastoma (Tan et al., 2023, 1-24). In summary, this research utilizing CRISPR technology for "cancer shredding" demonstrates a promising therapeutic strategy for eradicating glioblastoma cells while sparing normal ones, addressing hypermutated cancers' complexity, and offering the potential for overcoming treatment resistance.

Using CRISPR technology allows targeting of the vast non-coding genome and non-driver mutations, offering a broader range of therapeutic targets. Non-driver mutations are often overlooked in therapies because they don't initiate the cancer growth, but catalyze it once activated. The study identifies unique recurrent glioblastoma-specific guide RNAs (sgRNAs) personalized to patients' tumors, mainly in the non-coding genome and generated by TMZ mutations characteristic of hypermutated gliomas (Tan et al., 2023, 1-24). Overall, scientists can use this same data to help identify the cause and growth of other hypermutated cancers. Cancer shredding enables the selective elimination of hypermutated cancer cells through targeting therapy-induced (in this case TMZ) mutations, presenting an outlet for therapeutic development.

Treatment for Genetic Disorders

According to the World Health Organization (2023), approximately 10 out of every 1000 people suffer from genetic disorders. Common genetic disorders, such as sickle cell anemia, cystic fibrosis, Alzheimer's disease, and others associated with genetic mutations, often lack effective long-term treatments, leading to reduced life expectancy. Many of these disorders are heritable, meaning offspring have a chance of inheriting the disease based on specific recombinant factors. While treatments exist for some of these conditions, there is technically no direct cure, as the genetic mutations responsible are encoded into the genome. With the uprising of gene editing technology, scientists theoretically can inhibit or modify the gene sequences underlying these genetic disorders, potentially providing a cure. As discussed further in the regulatory landscape section, one gene editing therapy for sickle cell anemia, utilizing CRISPR technology, has been approved. This therapy involves removing bone marrow cells from the body and genetically altering them with CRISPR to produce a hemoglobin derivative that slows the sickling of blood cells. This treatment provided relief for 96.7% of the 29 subjects for at least

18 months (Stein, 2023). However, while promising, the therapy remains inaccessible to most patients due to its high wholesale price, ranging from 2 to 3.1 million dollars (Pagliarulo, 2023, para. 2). Another recent approval occurred in March 2024 for the treatment of Metachromatic Leukodystrophy (ML) in eligible children costing roughly 4.1 million dollars wholesale (Shaw, 2024). ML is a rare genetic disorder characterized by fat buildup in cells, primarily affecting spinal cord and brain nerve cells due to a deficiency in the enzyme sulfatide. This buildup damages the myelin sheath protecting nerves, resulting in impaired nerve function, motor control issues, sensory deficits, muscle stiffness, blindness, and seizures. Diagnosis in children often leads to early death due to disrupted developmental factors (Mayo Clinic, 2020). Lenmeldy™ is the first FDA-approved treatment for ML, developed by Orchard Therapeutic Gene Therapy using ex vivo therapy with stem cells. Stem cells are extracted from the body and genetically modified with a functional copy of the ARSA human gene to enhance arylsulfatase enzyme production to prevent excess fat buildup. The gene editing technique uses a lentiviral vector targeting specific parts of the stem cell genome. Lentiviral vectors are derived from retroviruses that target the human genome and using this viral technique, scientists were able to use the vector as therapy transportation to implement into the genome. Genetically corrected cells are then reintroduced into the body to slow or halt ML progression. Compared to untreated patients, children receiving Lenmeldy™ showed extended survival and improved cognitive and motor functions over a 12-year follow-up period (Orchard, 2024). While these newly FDA-approved gene editing therapies offer hope for genetic disorder treatments, their high costs exemplify the need for future research to develop more affordable options.

Literature Review: Ethical and Technological Dilemmas

Ethical Perspectives

Genome editing raises significant ethical considerations regarding the potential alteration of human nature. From a therapeutic and medicinal standpoint, the application of genome editing hinges on whether it interferes with established biological mechanisms, potentially resulting in unforeseen and possibly fatal consequences. Discussions, often in the context of eugenics, often center around whether modifying the human genome could create a predetermined life path, thereby infringing upon personal freedoms. There is concern about whether altering the genetic makeup of current society could diminish the diversity of individuals, affecting future recombination processes irreversibly. Alternatively, the occurrence of random, rapid mutations could lead to irreversible changes in phenotype, posing potential risks to humanity. These ethical dilemmas highlight the need for careful consideration and regulation of genome editing technologies (Joseph et al., 2022, p. 1-18). The ethical considerations surrounding genome editing, particularly in the context of potential alterations to human nature highlights the importance of carefully balancing therapeutic benefits against risks to established biological mechanisms and freedoms, necessitating intricate regulation to navigate these complex dilemmas.

As genetic engineering advances, starting from modifying viruses for vaccines to potentially applying germline edits to shape human traits across generations, ethical questions arise across bioethical, moral, and social realms. These issues pose potential barriers to the expansion of genetic engineering, meaning scientists must find a delicate balance between its applications, risks, and ethical considerations. Geneticists and bioethicists navigate the frame of genetic engineering by assessing its effectiveness, safety, and ethical policies, even when gene editing isn't yet a mainstream medical practice. According to researchers at Nova Southeastern University College of Osteopathic Medicine, the discussion dwindles to the philosophical,

theological, cultural, and public perspectives, alongside bioethical research focused on therapeutic legitimacy and consent (Joseph et al., 2022, p. 1-18). To gauge the current ethics surrounding recent gene editing advancements like CRISPR-Cas9, the research team conducted a scoping review. This review analyzed the ethical domain using computational methods to determine the consensus and implications of ongoing debates. By employing computational analysis and keyword searches such as "gene editing" and "ethics," the team gathered a plethora of relevant studies. These studies were then subjected to exclusion criteria, leading to a final analysis to determine an overarching consensus on the ethical perspectives and implications of gene editing advancements.

In the philosophical realm, the central concern revolves around the concept of dignity in genetic engineering. While some argue that this technology threatens the dignity of both science and human well-being, the majority believe that despite these concerns, we should not pause the development of genetic engineering. Instead, they argue that it holds the potential to enhance and improve ourselves. Much debate stems from the controversial case of the "CRISPR babies," genetically modified embryos altered by Dr. He Jiankui, which lacked proper safety and regulation protocols. In 2018 at the Second International Summit on Human Genome Editing, Dr. He Jiankui presented his study, shocking the scientist-filled crowd with the common consensus that his practice was “irresponsible human experimentation” (Stein, 2023, para. 2-3). While opinions vary, the common word is that altering human nature, especially without adequate safety and regulation, is not advisable. Theological perspectives on genetic engineering exhibit nuanced differences, requiring ongoing discussions and collaboration between scientists and religious experts to ensure alignment with religious principles. Public perspectives on genetic engineering vary by country, influenced by cultural norms and values. In the United

States, therapeutic applications of genetic engineering are generally acceptable, provided adverse effects are mild and treatable. However, there is significant apprehension about expanding beyond therapeutic uses, with a preference for strictly therapeutic applications in a survey of 1600 Americans asking if genetic engineering should expand into the realm of human enhancement. Lastly, research ethics is the tension between the desire to address potentially curable genetic disorders and the practical realities of access and affordability. The concern is that genetic editing technologies may emphasize existing social inequalities if only accessible to a select few. Additionally, ensuring the consent of females is a priority as obstetric issues are at large. Irreversible harm could result from inadequate trial oversight (Joseph et al., 2022, p. 1-18). As the field of genetic engineering progresses, it is important to strike a balance between scientific innovation and ethical considerations. Collaboration between scientists, bioethicists, policymakers, and the public is essential to ensure that genetic editing technologies are deployed responsibly and with due regard for human values and dignity.

Off-Target Edits and Prevention Strategies

The use of genetic engineering introduces the possibility of unintended off-target edits in the human genome, which can lead to adverse effects not initially intended and can catalyze the worsening of genetic malfunctions. Within the CRISPR-Cas9 mechanism, the protein Cas9 based on its evolutionary immune response patterns, may produce unexpected results and inefficiencies. The protein contains the molecular potential of interacting with non-target genetic sequences and causing cleavages, resulting in outcomes like genetic silencing or undesired gene expression (Guo et al., 2023). These off-target effects can include altered cellular processes, transcription errors, phenotypic changes, or increased activity in disease-associated genetic codes. Theoretically creating the issues that modern geneticists aim to resolve with CRISPR

technology. Efforts are currently underway to improve the accuracy of gene editing technology and identify off-target edits.

A new and promising genetic tool was created in response to the Off-Target edits manifested with CRISPR technology. CRISPRme is a genetic command line developed by InfOmics; a biotechnology company affiliated with the University of Verona in Italy. The team discusses the major mechanism behind CRISPR, utilizing guide RNA (gRNA) to direct a specific DNA sequence along with its designated effector protein. These effector proteins carry out specific functions in genome editing. These include but are not limited to nucleases for creating double-strand breaks, deaminases for accurate substitutions, and chromatin regulators for altering transcription. While off-target edits are still under research, geneticists can predict their locations by examining the homology of the spacers and PAM sequences (Cancellieri et. al 2023, p. 34-53). Spacers are customized RNA sequences complementary to specific target sequences within the genome. The Protospacer Adjacent Motif (PAM sequence) consists of adjacent sequences to the targeted DNA sequence, playing a crucial role in facilitating the binding of the CRISPR system to the target sequence and enabling genetic edits to be carried out effectively (Adebiyi, 2023). As research continues to refine our understanding of off-target effects and prediction methods, tools like CRISPRme hold promise for advancing the field of genetic engineering with greater accuracy, precision, and safety.

Previous computational tools and algorithms have strictly relied on identifying homologous sequences using reference genomes or human donor genomes to assess off-target effects. Analyzing population-based databases like the 1000 Genomes Project has highlighted how individual genetic variability affects the off-target landscape. However, since each person has a unique genome, off-target effects can vary significantly for each individual, even to the

extent of altering protospacers and PAM sequences differently, resulting in unplanned on and off-site edits and potentially rendering treatments ineffective or causing more harm. In response to these challenges, the comprehensive online tool CRISPRme was developed to oversee gRNA in CRISPR-based genome editing. Unlike earlier off-target algorithms, CRISPRme is capable of detecting haplotype-aware off-target sites, considering short indel variants, and offering customizable parameters such as mismatches, bulges, and specific Cas proteins and PAM sequences. CRISPRme allows users to incorporate their own genome annotations, enabling the detection of person-specific off-target sites or cell-specific chromatin features. Thus providing valuable insights before clinical genetic trials along with percentages for error. InfOmics demonstrated the usage of CRISPRme by conducting a comprehensive analysis of off-target potentials in clinical trials for β-thalassemia and Sickle Cell Disease, revealing how genetic variations influence off-target potentials on an individual basis. In the study focusing on β-thalassemia and sickle cell disease, researchers found that patients with sickle cell disease of African descent carrying a specific allele had approximately a 10% likelihood of off-target cleavage (Cancellieri et al., 2022, p. 34-53). This off-target cleavage was not detected in other computational analyses, most likely due to a lack of specificity in the analyzed genome. This displays the effectiveness of the new technology and the importance of implementing variant-specific off-target analysis, as off-target edits are not equally distributed among ancestral groups. Furthermore, the study analysis shows that off-target variants may be individualized as well rather than just on a population basis. CRISPRme was concluded as an effective tool in identifying variant-specific off-target editing sites which other computational technologies miss. While the new technology aids in the detection of off-target sites, it does not prevent off-target edits but rather facilitates their identification before editing. According to the research team, to

mitigate risks, it is essential to combine CRISPRme with highly specific gene editing tools and assess allele-specific off-target effects in relevant primary cells. It's crucial to address other techniques to minimize off-target edits before widespread application. [Link to CRISPRme: http://crisprme.di.univr.it/index] (Cancellieri et al., 2022, p. 34-53). The study and application shows the importance of implementing variant-specific off-target analysis, as off-target edits may not be equally distributed among ancestral groups, individual genomes, and other factors.

Literature Review: Regulatory Landscape

Current United States Policy

Genetic engineering in the United States is regulated through a comprehensive framework of policies established by the Federal Food and Drug Administration (FDA), covering various aspects such as the type of genetic engineering (germline vs. somatic), age groups involved, clinical trial protocols, and medication delivery methods. The emergence of the genetic revolution, along with the eagerness of clinical geneticists to address lethal genetic conditions, has led to intense debate and policy development aimed at expanding the use of genetic tools, particularly CRISPR, and initiating clinical trials.

The FDA prioritizes regulating human gene therapies for genetic diseases, considering the diverse techniques and potential outcomes involved. Germline gene editing, which involves permanently altering the genetic makeup of an individual and any potential offspring, raises significant ethical concerns, including fears of misuse (e.g., "designer babies") and potential biological complications like mosaicism. Consequently, **the U.S. government has refrained from allocating funds to germline gene editing projects due to ethical and biological apprehensions.** In 2019, the U.S. government withheld funding for germline gene editing

projects following the National Institutes of Health's consensus that such endeavors create significant ethical and biological challenges (Collins, 2019). This stance has stayed unchanged, potentially influenced by the 2020 Covid-19 pandemic. Notably, this decision was reinforced by the widely condemned experiment conducted by scientist He Jiankui in China (Collins, 2019). Jiankui's attempt to create HIV-resistant embryos, lacking medical necessity and ethical oversight, drew strong condemnation from global scientific communities. Over 100 Chinese biomedical researchers criticized Jiankui's experiment for its recklessness and lack of consent, diminishing the reputation of genetic biomedical research in China. After multiple calls of action to Chinese authorities, Jiankui faced legal repercussions for his actions, highlighting the importance of oversight and adherence to ethical guidelines in gene editing research. Despite initial reports suggesting the twins born from the experiment were healthy, the long-term effects of germline gene editing on individuals and future generations remain largely uncertain, emphasizing the need for continued debate and regulation in the field. Discussions of a national moratorium on germline gene editing were conducted based on the public opinion of He Jiankui's actions, yet never enforced (Collins, 2019). The incident highlights the importance of transparent and responsible gene editing practices, prompting ongoing discussions among scientists and policymakers on ethical gene editing applications and regulatory frameworks. While Jiankui's experiment occurred in 2018, its ramifications continue to shape international attitudes towards gene editing, including the U.S. government's decision not to fund germline gene editing projects since the 2019 decision. While the law doesn’t directly state that germline editing is illegal and private investors still could fund these experiments; the general consensus is that private investors and scientists are resistant to the idea based on the other potential legal repercussions that may occur from it.

Somatic gene editing, or therapeutic editing, involves altering the genetic code within the non-reproductive cells of an individual. Unlike germline gene editing, which modifies genetic material in reproductive cells and can be inherited, somatic gene editing affects only the treated individual and is not passed to future generations. **In the United States, somatic gene editing can be approved and funded if it adheres to FDA protocols, similar to other therapeutic products.** Currently, the FDA primarily focuses on somatic gene editing due to its potential to treat chronic genetic diseases like sickle cell disease. In December 2023, the FDA approved the first gene therapies for sickle cell disease. Sickle cell disease is when a mutation in the proteins of a red blood cell causes a lack of blood flow to tissues and organs around the body. It can be fatal and cause other issues like severe pain and organ damage (VOEs and VOCs). One therapy, Casgevy™, modifies patients' blood cells (hematopoietic stem cells which produce all blood cells) using CRISPR Cas9 to increase hemoglobin production which would then increase oxygen production (Vertex Pharmaceuticals Incorporated, 2024). Another therapy, Lyfgenia, uses a viral vector to alter blood stem cells to produce HbAT87Q, a derivative of hemoglobin that is less likely to sickle which could reverse the effects of the disease.

Both therapies utilize ex vivo editing, where cells are removed, edited externally, and reintroduced. According to Peter Marks, the director of the FDA's Center for Biologics Evaluation and Research, "These approvals represent an important medical advance with the use of innovative cell-based gene therapies to target potentially devastating diseases and improve public health. Today's actions follow rigorous evaluations of the scientific and clinical data needed to support approval, reflecting the FDA's commitment to facilitating the development of safe and effective treatments for conditions with severe impacts on human health" (FDA News Release, 2023, para. 3). This approval highlights how the FDA is applying its principles to

approve therapeutic gene editing treatments that align with specific ethical and biological concerns. In January 2024, the FDA released a guidance document focusing on the development and evaluation of human Genome-Editing products (GE). This guidance primarily addresses somatic and therapeutic edits, with minimal discussion on germline editing, emphasizing that GE products must not cause heritable germline edits. The guidance outlines key considerations such as potency testing, nonclinical studies, clinical trial design, and communication with regulatory authorities. Potency testing for ex vivo-modified human GE drug products is emphasized, highlighting the importance of assessing both cell properties and intended downstream biological modifications resulting from genome editing. While confirmation of genetic sequence modifications serves for early-phase studies, potency assays should evaluate downstream biological modifications for marketing application support. Additional testing, including surrogate potency tests, is warranted for products from donors if supported by correlating data. In nonclinical studies, proof-of-concept evaluations are recommended using in vitro and in vivo models to assess activity and safety, including the identification of on- and off-target editing events. Clinical study design should prioritize appropriate patient populations and safety monitoring, with long-term follow-up for up to 15 years. Early communication with regulatory authorities is encouraged to discuss product-specific considerations and obtain regulatory advice. The guidance emphasizes the importance of consent and transparency in GE products and testing, especially in children. This ensures that both parties are aware of any negative outcomes and provides full details on potential biological changes (FDA Guidance for Industry, 2024). Overall, the guidance highlights the importance of thorough characterization, safety assessment, and transparent communication throughout the development and evaluation of human genetic engineering products. With this guidance, private investors and gene editing (GE) developers

now have a detailed outline of what is necessary for a product to be approved for clinical use. Additionally, it provides the FDA with a clear framework outlining the details needed for approving gene editing products (FDA Guidance for Industry, 2024). This exemplifies how the FDA prioritizes safety and transparency in the growth of gene therapy products in society. The FDA's Center for Biologics Evaluation and Research (CBER) Office of Therapeutic Products (OTP) conducted a virtual public webinar on Thursday, February 29 at 1:00 pm, addressing newly finalized guidance regarding the development of human gene therapy products involving human genome editing. This guidance aims to aid industry stakeholders by offering recommendations for content to be included in investigational new drug (IND) applications, as well as guidance on product design, manufacturing, and testing protocols (FDA CBER, 2024). Additionally, other regulatory workshops are conducted frequently, many of which highlight specific concerns like off-target effects or other genetic engineering variables. It's important to note that these workshops do not represent final opinions from the FDA, indicating that policy can be subject to change based on arguments and evidence presented. Overall, the FDA maintains a highly restrictive and regulated approach to somatic/therapeutic gene editing but remains open to approval as long as all legal, consensual, medical, and other requirements are met.

Conclusion

In conclusion, the rapid advancement of gene editing technology holds potential for transforming medical treatments, particularly in the fields of cancer therapy and genetic disorders. With these scientific breakthroughs come significant ethical considerations and the

need for a comprehensive regulatory framework to ensure responsible and safe implementation. Ethical perspectives surrounding gene editing are based on concerns about human dignity, diversity, theological implications, public acceptance, and research ethics. These perspectives highlight the importance of engaging in bioethical discussions and collaboration among scientists, policymakers, and the public to navigate this landscape. Furthermore, the emergence of off-target effects in gene editing highlights the importance of developing preventive strategies, such as the innovative genetic tool CRISPRme, to help prevent unintended consequences and ensure the safety of gene therapies. From a regulatory standpoint, the FDA plays a crucial role in overseeing gene editing research and development, prioritizing safety, transparency, and thorough characterization whilst collaborating with scientists to create the best policy for safety and technological advancements. While somatic gene editing has seen approvals for clinical use, germline gene editing remains an issue due to ethical concerns. For the future, it is essential to strike a balance between scientific innovation and ethical considerations, ensuring that gene editing technologies are deployed responsibly and with due regard for human values and dignity. Continued collaboration, transparency, safety implementations, and adherence to regulatory guidance will be crucial in the expansion of gene editing. As a result of the Genetic Revolution, advancements in gene editing technology have sent medical science to an all-time peak, enabling precise modifications to the human genetic code and addressing underlying issues to change the landscape of therapeutic interventions.

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