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Beyond the hype: A critical review of crispr-Cas9 gene editing therapies

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Abstract

CRISPR-Cas9 gene editing has emerged as a revolutionary tool with the potential to reshape medicine. However, critical evaluation is necessary to navigate beyond the hype and assess its true therapeutic potential. This review examines the background, promise, and challenges of CRISPR-Cas9 therapies. It explores its simplicity, efficiency, and transformative impact on biological research. While acknowledging the excitement, the review emphasizes the need to address ethical concerns, off-target effects, delivery issues, and long-term safety. By critically appraising the efficacy, safety, and ethical considerations, this review aims to inform stakeholders about the realities of implementing CRISPR-Cas9 in clinical practice. Furthermore, it explores future directions in research and emphasizes the importance of responsible development and implementation to maximize benefits while minimizing risks.

Keywords: CRISPR-Cas9; Gene Editing; Therapeutic Potential; Ethical Concerns; Off-Target Effects

1. Introduction

Since its emergence as a revolutionary gene editing tool, CRISPR-Cas9 has ignited widespread excitement and garnered immense attention from the scientific community, policymakers, and the public alike. Promising the potential to address a myriad of genetic disorders and reshape the landscape of medicine, CRISPR-Cas9 has been hailed as a game-changer in the field of biotechnology(1). However, amidst the fervor and anticipation surrounding CRISPR-Cas9, there exists a pressing need for a critical examination of its therapeutic applications to navigate beyond the hype and discern the realities of its efficacy, safety, and ethical implications.

The background of CRISPR-Cas9 lays the foundation for understanding its transformative potential and the challenges associated with its implementation(2). Discovered as a natural defense mechanism in bacteria against viral infections, CRISPR-Cas9 enables precise manipulation of DNA sequences, offering unprecedented opportunities for targeted modifications within the genome.

The rise in excitement and adoption of CRISPR-Cas9 has been meteoric, driven by its simplicity, efficiency, and versatility. Unlike previous gene editing techniques, CRISPR-Cas9 allows researchers to edit genes with unprecedented precision, speed, and cost-effectiveness. Its widespread adoption across diverse fields of biological research, ranging from basic science to therapeutic applications, underscores its transformative potential. CRISPR-Cas9 has catalyzed a paradigm shift in our ability to study and manipulate genetic information, opening new avenues for understanding the genetic basis of diseases and developing targeted therapies. However, the enthusiasm surrounding CRISPR-Cas9 has also been accompanied by a growing recognition of the need for critical evaluation. While the promises of CRISPR-Cas9 are undeniably tantalizing, there are significant challenges and limitations that must be addressed. Ethical concerns

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regarding the potential misuse of CRISPR-Cas9, such as germline editing and unintended off-target effects, have sparked widespread debate and calls for regulatory oversight. Moreover, the translation of CRISPR-Cas9 from bench to bedside faces formidable hurdles, including delivery issues, immune responses, and long-term safety considerations.

This critical review seeks to go beyond the hype surrounding CRISPR-Cas9 gene editing therapies and provide a comprehensive assessment of its current state. By critically examining the existing literature, clinical trials, and real-world applications of CRISPR-Cas9, this review aims to elucidate the opportunities and challenges inherent in harnessing this technology for therapeutic purposes. Through a rigorous evaluation of the efficacy, safety, and ethical implications of CRISPR-Cas9 gene editing therapies, this review seeks to inform stakeholders and policymakers about the realities of implementing CRISPR-Cas9 in clinical practice.

In pursuit of this objective, the review will adopt a multidisciplinary approach, drawing upon insights from genetics, molecular biology, bioethics, and regulatory science. By synthesizing evidence from preclinical studies, clinical trials, and ethical analyses, this review will provide a balanced assessment of CRISPR-Cas9 gene editing therapies. Additionally, the review will explore emerging trends and future directions in CRISPR-Cas9 research, including advancements in delivery methods, genome-wide editing strategies, and precision medicine applications.

While by examining the opportunities and challenges associated with CRISPR-Cas9 gene editing therapies, this review aims to foster informed discussions and decision-making regarding the responsible development and implementation of this technology(3). Ultimately, by transcending the hype surrounding CRISPR-Cas9, we can navigate a path forward that maximizes its benefits while minimizing potential risks and ethical concerns.

1.1. History

- **1993: The principles of CRISPR were discovered.**

Many people believe that Jennifer Doudna and Emmanuelle Charpentier invented CRISPR. However, Francisco Mojica discovered the basic idea behind CRISPR—which stands for "Clustered regularly interspaced palindromic repeats"—when studying bacteria in the Santa Pola marshes. Mojica noticed that certain DNA segments in the bacteria repeated repeatedly, with regular gaps in between. Mojica spent the next ten years delving further into these repeats, and in 2003 he made the crucial finding that the DNA repeats corresponded with fragments of the viruses that were attacking the bacteria.Over the following ten years, this idea sparked a plethora of additional breakthroughs in DNA research that elevated the technology of CRISPR gene editing to the forefront of genetics research. (4)

- **2012: A tool for CRISPR genome engineering was discovered.**

Emmanuelle Charpentier, Jennifer Doudna, and their researchers clarified the molecular workings of CRISPR technology in 2012. CRISPR has opened up countless possibilities in the fields of biomaterials, agriculture, medicine, and other fields by precisely cutting targeted regions of DNA. Bacterial nuclease, a CRISPR-associated protein, snips off bits of DNA from invasive viruses as part of the CRISPR-Cas9 adaptive immune system. The chopped-off DNA fragment is stored in memory to help fend off infections in the future. It is possible to modify eukaryotic DNA using the CRISPR-Cas9 system by creating guide RNA that is complementary to the target sequence. A20-base pair protospacer motif with flanking homology to the cut site of interest is present in the guide RNA. This guide RNA's protospacer motif is bound by Cas9, and Cas9 then binds to the target site. Next, Cas9 attaches itself to the genomic DNA's protospacer adjacent motif (PAM) and catalyzes a double strand break (DSB) three base pairs upstream of the PAM. HDR will happen if a homology arm is included with the CRISPR-Cas9 cassette; if not, the cell will use NHEJ to make tiny indels at the cut spot of interest. CRISPR technology is being applied to a wide range of projects, including the development of hornless cows, obesity treatment, and cancer treatments. Every day, new findings in this sector are being made, whether they are related to diagnosis, treatment, or something else entirely. (5)

- **2013: Eukaryotic Cells Displayed the Utility of CRISPR**

The work done by Feng Zhang to show how CRISPR may be used to genetically modify eukaryotic cells outside of the bacterial realm was equally important. His lab uses optogenetics—a technique in which light is used to regulate genetically engineered neurons in order to find medicines to cure brain disorders—to investigate the workings of the brain using CRISPR. (6)

- **2015: CRISPR Editing of Human Embryo**

At Sun Yat-Sen University in Guangzhou, Junjiu Huang employed CRISPR in 2015 to modify human embryos. This was first turned down by Western science journals for violating scientific ethics, but it eventually found a home through alternative channels. Huang's attempt to correct a gene defect causing a blood disorder was deemed unethical due to his alteration of the germline cells that influence inheritance, and it quickly became a contentious issue. This occurred three years prior to any regulatory authority formally approving CRISPR human studies. (7)

- **2018: saw the approval of the first CRISPR human trials.**

A collaborative effort between Vertex Pharmaceuticals and CRISPR Therapeutics has cleared the initiation of clinical trials for an investigational treatment for beta-thalassemia, a blood condition. If effective, this treatment could put a stop to sickle cell anemia and occurrences of this illness. It would alter the way that diseases are treated in people by opening the door for more CRISPR therapies. (8)

- **2020: The Year of CRISPR:**

Nobel Prize, Clinical Trial Success, and More In the summer of 2020, information about the CRISPR clinical trials started to dribble in. Victoria Gray was the first patient to receive treatment for sickle cell disease, and her encouraging outcomes began to garner media attention. Less than half a year later, at the December 2020 ASH meeting, data was presented indicating that ten patients had made significant progress—seven of them were receiving treatment for beta-thalassemia, and the remaining three for sickle cell anemia. These patients all saw significant improvements in their blood levels of fetal hemoglobin, experienced periods of pain alleviation, and were no longer in need of blood transfusions. In October 2020, CRISPR gained more notoriety when it was revealed that Jennifer Doudna and Emmanuelle Charpentier had finally been awarded the Nobel Prize in Chemistry for creating CRISPR. (9)

1.2. Comparison of Crispr with Genome Editing Tools

Table 1 Detailed comparison of genome editing tools, including CRISPR

Feature	**Mega nucleases (MNs)(10)**	**Zinc Finger Nucleases (ZFNs)(10)**	**Transcription Activator-Like Effector Nucleases (TALENs)(10)**	**Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)**
Origin	Natural (homing endonucleases) or engineered	Artificial	Artificial	Natural (bacterial defense system)
DNA Recognition	Long target sequences (12-40 bp)	Engineered zinc finger motifs (3-6) targeting 9-18 bp	TALE repeat arrays with RVDs for one-to-one base recognition	Guide RNA targeting 20-ish bp sequence
Structure	Two αββαββα domains	FokI nuclease domain fused to ZF DNA-binding domain	FokI nuclease domain fused to TALE DNA-binding domain	Cas9 nuclease and guide RNA molecule
Cleavage Mechanism	Introduce DSBs at specific sites	FokI dimerization required for DSB introduction	FokI dimerization required for DSB introduction	Cas9 cleaves DNA based on guide RNA sequence
Overhang	4-nt 3' overhang	3-nt 5' overhang	Variable overhang depending on FokI variant	Blunt ends
Advantages	High specificity (if engineered), small size, non-repetitive sequence	Modular design, various delivery methods	Highly customizable DNA recognition, reduced off-target activity compared to ZFNs	Easy to design and use, highly efficient, inexpensive

Disadvantages	Laborious development and optimization, limited target sites	Low efficiency, context-dependent DNA recognition, off-target activity	Complex assembly, potential immunogenicity from FokI, off-target activity	Potential off-target effects, ethical considerations
Applications	Gene editing, research on genome function	Gene therapy, correction of genetic mutations	Gene editing, research on gene function	Gene therapy, research on gene function, drug discovery

2. Pros of CRISPR

- **Ease of develop and Use:** CRISPR is comparatively simple to develop and use when compared to other genome editing instruments like ZFNs and TALENs. Researchers and therapists can more easily utilize the guide RNA molecule since it is easily programmable to target a particular DNA sequence.(11)
- **High Efficiency**: CRISPR has a high editing efficiency, which enables it to insert the necessary modifications accurately and successfully into a target gene. Because of this, it's an effective tool for gene therapy and other uses.(12)
- **Versatility:** CRISPR is an adaptable instrument with a wide range of genome editing uses. Because it may be used to add, remove, or alter genes, it can be tailored to a variety of scientific and medical applications.(12)
- **Cost-Effectiveness:** In comparison to other genome editing technologies, CRISPR is a reasonably priced technology. This increases its accessibility for a larger group of academics and organizations.(12) **Improved Specificity:** Current research aims to reduce off-target effects—a situation in which CRISPR edits parts of the genome that are not intended—by making the technology more specific. Even while CRISPR has demonstrated promise in this regard, research is still ongoing to achieve even more specificity.
- **Many Delivery Techniques:** Researchers are working on a number of techniques to introduce the guide RNA and Cas9 protein, two components of CRISPR, into cells. This makes it possible to use CRISPR in a variety of contexts, such as gene therapy, where the tool must be inserted into the patient's cells.

3. Mechanism of the Crispr-Cas9 Gene-Editing system

Systems utilizing CRISPR-Cas are separated into two groups. Types I, III, and IV are found in the class 1 system, while types II, V, and VI are found in the class 2 system.(13) Whereas the class 2 CRISPR-Cas system employs a single Cas protein with several domains, the class 1 system uses a complex of multiple Cas proteins. Because of its simplicity and ease of use, the class 2 CRISPR-Cas system is therefore preferred for gene-engineering applications.(14)The type II CRISPR-Cas9 system is the most extensively utilized and researched among the several class 2 CRISPR-Cas systems. In this system, the Cas9 protein regulates spacer acquisition and defense while CRISPR spacers guide the system toward the target. Three phases of activity are present in natural CRISPR systems: adaptation, expression, and interference.(14-17)Foreign DNA fragments (also known as protospacers; around 30-45 nucleotides) from invasive plasmids or viruses are added to CRISPR arrays as new spacers during the adaptation stage. The proto-spacer-adjacent motif (PAM) is used to select protospacers from the foreign DNA. Subsequently, fresh spacers offer memory specific to their respective invasive plasmids or viruses. Pre-CRISPR RNA (pre-crRNA), which is subsequently converted to mature CRISPR RNA (crRNA), is produced during the expression stage of the CRISPR array.(18, 19) A transcribed spacer and a conserved repeat sequence that are complementary to the foreign DNA are present in every crRNA. Each crRNA correlates to an invasion sequence, therefore a pool of crRNAs can target several gene elements.(20) During the interference step, Cas9 cleaves the matched DNA while crRNAs serve as a guide to precisely target the PAM. The sgRNA-Cas9 complex binds to the target DNA in the type II CRISPR-Cas9 system to ensure that the Cas9 cuts both strands of the DNA, preventing the spread of foreign DNA. The most widely utilized CRISPR gene-editing technology is Type II CRISPR-Cas9, which is commonly referred to as CRISPR. Scientists have successfully shown how to modify the genome of mammalian cells by engineering the type II CRISPR system. Ruv C and HNH are the two nuclease domains that the endonuclease Cas9 protein possesses.(21)DNA strands that are complementary can be broken by the HNH domain, but non-complementary DNA strands are broken by the Ruv C domain. The trans-activating crRNA (tracrRNA) and crRNA make up the sgRNA. A corresponding sequence to the tracrRNA and a 20-nt protospacer element are present in the crRNA. Double-stranded breaks (DSBs) are made at specific locations in the genome by the CRISPR-Cas9/sgRNA complex, which is formed when the tracrRNA hybridizes to the crRNA and binds the Cas9 enzyme. Normally, the dual-tracrRNA: crRNA is created as a single-strand sgRNA with two essential segments: a guide sequence at the 5' end that binds the target DNA sequence, and a duplex RNA structure at the 3' end that binds Cas9. This two-part technique is straightforward but effective. After sgRNA identifies a particular DNA sequence in the genome, Cas9 cleaves the sequence like a pair of scissors. The PAM

site is cleaved 3–4 nucleotides upstream by the Cas9 protein. One of two repair pathways—Homology Directed Repair (HDR) or Non-Homologous End Joining (NHEJ)—will begin once DSBs are generated. The insertion/deletion (In Del) produced by the NHEJ repair pathway frequently results in frame shifts and/or premature stop codons inside the open reading frames (ORFs) of target genes. On the other hand, the HDR pathway requires a donor DNA template in order to repair the DSBs. Using a donor DNA template, the exact DNA sequences are inserted into the target spot. Because gene replacement or knock-in is typically less effective than gene knock-out, the HDR process is significantly less effective than the NHEJ mechanism. Because CRISPR-Cas9 can directly edit mutations linked to disease, it has enormous promise for the treatment of genetic disorders. Significant efforts have been made to enhance the CRISPR-Cas9 system's specificity, gene-editing effectiveness, and delivery efficiency. Consequently, CRISPR-Cas9 has emerged as a groundbreaking genome-editing instrument with an extensive range of therapeutic uses.

4. Approaches for Delivering Crispr/Cas9 *In-vivo*

The use of the CRISPR system is hampered by the Cas9 protein's introduction into the cytosol and then the cell nucleus. There are multiple formats (or delivery cargos) available for the CRISPR/Cas9 system, each with its own set of advantages and disadvantages. Notably, viral vectors—species created by nature to deliver genetic material to be produced in the cell—can be modified to include plasmids for the Cas9 protein and its corresponding gRNA. The CRISPR/Cas9 system has been delivered mostly using viral techniques; in fact, so much so that delivery formats are classified as either "viral" or "non-viral." Applications of viral methods for delivering gene editing machinery, such as animal model development and ex vivo transfection, have proven successful. The primary reason for their efficacy is the innate capacity of viral vectors to incorporate exogenous genetic material into the cell, typically leading to high transfection efficiency. However, there are major practical and safety obstacles to viral techniques. Alternative methods have been investigated as vehicles to introduce the CRISPR/Cas9 system into cells, mostly due of these rationales. These artificial "carrier vehicles" are designed to contain and transport the CRISPR/Cas9 machinery in non-viral forms. The CRISPR/Cas9 machinery is available in non-viral forms like as mRNA-based, plasmid-based, and complexed Cas9-RNP.Few of the methods created to bring the CRISPR/Cas9 machinery into the cell, however, are suitable for in vivo use since the delivery vehicle and its cargo are not stable or biocompatible. Numerous strategies that show promise for systemic application and translatability have been used to treat hepatic disorders. Because of its function in the mononuclear phagocyte system (MPS), this target is useful, but it restricts the tissue specificity of these systems for diseases that are not liver-specific. Because there aren't many systemic delivery options, the problem of disease tissue specificity of systemically-delivered medicines is still a major barrier for CRISPR/Cas9 delivery systems, but it is still in its early stages. The advantages and disadvantages of some of these potentially clinically transferable strategies, as well as each one's potential for clinical transferability, will be covered in the sections that follow.

4.1. Viral delivery methods

The most popular techniques for delivering CRISPR/Cas9 are viral ones. Using lentiviral, adenoviral (AV), and adeno-associated viral (AAV) approaches, genome-editing agents have been widely used to integrate DNA encoding CRISPR/Cas9 machinery into the host genome and express the CRISPR/Cas9 machinery extra chromosomally. Since 1989, more than 2,000 AV-related clinical trials have been approved. AV vehicles are efficient and well-researched transduction agents for gene transfer. Both dividing and nondividing cells can be transduced by AVs, which often continue to express extra chromosomally instead of integrating into the host genome. These vectors usually cause a strong immune response in the host. Despite this immunogenicity, in vivo proof-of-concept studies have shown that the PTEN gene may be edited with >22% efficiency. In vivo proof-of-concept studies have shown that systemic injection can alter the PTEN gene in hepatocytes with >22% efficiency, despite this immunogenicity. However, within a few weeks, mice receiving this treatment also displayed severe hepatomegaly and a strong immunological response, likely due to the viral delivery components being produced in the cell. Ehrke-Shulz et al.'s latest study showed how to transport the CRISPR/Cas9 machinery utilizing a high-capacity AV vector that is newer and doesn't contain any viral genes. These CRISPR/Cas9 constructs were delivered into immortalized and primary cell lines with highest indel activities up to 93%, targeting the human papillomavirus (HPV) oncogene E6, the dystrophin gene associated with DMD (Duchenne muscular dystrophy), and the HIV co-receptor C-C chemokine receptor type 5 (CCR5).Although this strategy seems promising, it hasn't been used in vivo until lately. A non-specific yet extremely effective way to integrate a supplied gene into the host genome is by lentiviral vectors. The dangers of non-specific integration include insertion into random regions of the host genome and the potential for insertion into important host genes, which can result in insertional mutagenesis. An extreme illustration of the risks connected to lentiviral-based CRISPR therapeutics, particularly in vivo, is the potential for cancer resulting from random integrations of genes close to a protooncogene. To get around these problems, non-integrating lentiviral vectors have been created, albeit their effectiveness has reduced in contrast. Currently, lentiviral techniques are best suited for in vitro applications because of these drawbacks. Nevertheless, a number of studies have shown that lentiviral CRISPR editing is specific in vivo, particularly when it comes to the targeted editing of the tumor-

suppressor genes KrasLSL−G12D/+ and Trp53fl/fl Normally, the dual-tracr RNA: crRNA is created as a single-strand sgRNA with two essential segments: a guide sequence at the 5' end that binds the target DNA sequence in mice that cause lung cancer. This investigation showed that a variety of adenocarcinomas might be produced on purpose with no discernible off-target effects. AAV-based gene delivery techniques, in contrast to lentiviral and AV methods, allow for the regulated integration of supplied CRISPR genes into the mammalian cells' AAVS1 (adeno-associated virus integration site 1) locus. These approaches have a broad tropism and the capacity to transfect both dividing and non-dividing cells. Because AAV-based systems prevent a large portion of the toxicity associated with random integration, they are the safest viral delivery methods and are hence more suited for in vivo application. In a fascinating study, TabeBrodgar et al. employed an AAV vector to insert CRISPR elements into mice, triggering NHEJ of the dystrophin gene in muscle tissue to fix the mutant dystrophin gene in murine models of DMD. In these investigations, two distinct AAV vectors were used to deliver spCas9 and sgRNA independently to DMD model postnatal mdx mice by intramuscular, retro orbital, and intraperitoneal (IP) injections. The scientists reported a reading frame restoration with restored synthesis of dystrophin, albeit in a modified version that enhanced skeletal muscle performance more than the wild type (wt.) protein, through targeted knock-out of the faulty exon. Systems based on AAVs have inherent problems with package dimensions. Gene packaging by AAV systems is limited to around 4.7 kilobase pairs (kbp). Given that the Cas9 gene is 4.3 kbp in size, two distinct AAV vectors must be used to transport the Cas9 gene and sgRNA. This restriction severely reduces flexibility and brings up further questions about the incorporation of donor DNA or additional genes into the vector. Smaller Cas9 variants (such as Streptococcus aureus Cas9, SaCas9) that enable the packaging of genes encoding both Cas9 and sgRNA into a single vector have been used to get around this packing size restriction. However, it has been demonstrated that saCas9, as opposed to spCas9, is more immunogenic in animals, which further impedes the therapeutic use of this approach. (22)

4.1.1. Strategies for CRISPR/Cas9 based on plasmids

The CRISPR/Cas9 machinery can be introduced into cells by an appealing non-viral method called delivery of DNA encoding the Cas9 protein. Despite worries about unintended and perhaps adverse effects to the host, constant expression of the Cas9 protein does enable greater editing efficiency than other methods. The production of DNA is well-documented and reasonably simple. The material itself is robust and thermostable. Though theoretically similar to viral-based methods, synthetic vector-based gene delivery circumvents the problems related to viral material introduction into the cell. The CRISPR/Cas9 technology was first reported to be delivered systemically to adult mammalian tissues in 2014 by the Anderson lab. To demonstrate the possibility of treating human genetic diseases, the researchers used a mouse model of hereditary tyrosinemia that contained a point mutation (G->A) in fumarylacetoacetate hydrolase (Fah), a crucial enzyme in the tyrosine catabolic pathway. The CRISPR-Cas9 system's components were created and supplied in plasmid form via hydrodynamic injection. However, because of the poor expression of the wt.-Fah protein (~1/250 hepatocytes), the correction rate was insufficient for clinical translation, and it was speculated that this correction also caused concurrent weight gain. Plasmid-based CRISPR/Cas9 delivery needs a delivery mechanism that can rival viral vectors' high transfection efficiencies in order to be a practical choice. An artificial virus that targets the nucleus and is multifunctional was created in order to deliver a plasmid expressing Cas9 and sgRNA, while avoiding the difficulties associated with viral gene integration. Without the need for an extra nuclear localization signal (NLS), this synthetic viral vehicle promoted plasmid entry into the nucleus and expedited endosomal escape. Through dual-receptor mediated endocytosis, this vehicle was able to effectively disrupt the MTH1 gene (reducing gene expression in vitro by approximately 80%) and concurrently reduce tumor size in ovarian cancer patients. Recent advances in gene editing research have brought the difficulty of organ-specific targeting of CRISPR/Cas9 delivery vehicles to the fore, and its future therapeutic application depends heavily on it. A recent work showed that selective tumor selectivity in both orthotopic Accepted Manuscript osterosarcoma and lung metastasis could be achieved by functionalizing a cell-specific aptamer (LC09) onto a lipopolymer moiety. The ability to incorporate tissue- or cell-specific targeting into the plasmid itself is one possible benefit of plasmid-based delivery. Tissue targeting for targeted distribution to macrophages by incorporating a CD68 promoter onto the Cas9 expression plasmid. These creations were administered systemically through injection after being encapsulated into polymeric nanoparticles aided by cationic lipids. It was demonstrated that the netrin-1 protein was down regulated, which reduced the symptoms of type 2 diabetes (T2D) and concurrently decreased the release of other inflammatory cytokines linked to macrophages, such as TNF-α and IL-6. Furthermore, it is important to note that the most used cationic polymer for plasmid-based delivery is polyethyleneimine (PEI).Zuckermann et al. provide an illustration of PEI-mediated transfection. The authors of this study employed a PEI-CRISPR/Cas9 technique to delete one (Ptch1) or several tumor suppressor genes (Trp53, Pten, Nf1) in the rat brain through somatic gene transfer using distinct CRISPR plasmids. (23)

4.1.2. mRNA-based delivery

Another popular method for introducing the CRISPR machinery into the cell is the delivery of mRNA encoded with Cas9. In contrast to gene-based delivery techniques, mRNA-based approaches have a temporary effect, resulting in the nuclease's eventual elimination from the cell and avoiding the dangers of integration into the host genome. Since mRNA may be transcribed in a matter of minutes, mRNA-based techniques also enjoy the advantage of a rapid effect time. On the other hand, the intrinsic stability of mRNA and the need for separate delivery of each component severely restrict this delivery strategy. A recent publication from D.G. Anderson's group described a combinatorial delivery strategy that used AAV delivery of the sgRNA/HDR template and lipid-mediated delivery of Cas9 mRNA. In order to repair the Fah gene, these vectors were also given to a mouse model of hereditary tyrosinemia. This study reported correction in >6% of hepatocytes by systemic injection. This approach did depend on viral co-delivery to enhance mRNA delivery, although being quite successful. There are other obstacles to mRNA-based delivery methods' effectiveness than this one. Compared to other forms of genetic material, RNA is more brittle and frequently degrades too soon. This instability is particularly troublesome for sgRNA that is supplied before Cas9 complexation. It was postulated that sgRNA was severely degraded during mRNA translation, hence impeding the effectiveness of editing. Additional research revealed the advantages of synthetic sgRNA modifications, such as adding phosphorothioate bonds and altering the RNA 2'OH group to 2'OMe and 2'F, in order to increase efficiency by boosting sgRNA stability. This study, which instead focused on NHEJ-mediated correction of Fah mutant mice, found that >80% of hepatocytes underwent editing, as opposed to ∼40% when natural RNA was used. This is a practical way to get around the stability problems that occur with RNA-based CRISPR/Cas9 delivery. Synthetically modified sgRNA containing mRNA encoded with spCas9 was packaged into a lipid nanoparticle carrier in a related study conducted by Finn et al. After systemic injection, the mice's transthyretin (Ttr) gene was effectively knocked down in the liver, resulting in a >97% decrease in blood protein levels that stood the test of time for up to a year. Even though co-delivery has been successful in some studies (e.g., Finn's), mRNA-based techniques are usually still constrained by the requirement for several deliveries; in most cases, Cas9 mRNA is supplied independently of a second vector containing the sgRNA, and in the case of HDR, a homologous DNA template. Nonetheless, with a single zwitterionic amino lipid (ZAL) delivery vector, Miller et al. recently announced the first successful non-viral co-delivery of Cas9 mRNA and sgRNA. The amine-rich linker region and zwitterionic head group were designed into these vehicles. The mRNA encoding the CRISPR mechanism was coupled onto this vehicle using a sequence of hydrophobic tails. This work revealed up to 95% protein knockdown in vitro and intravenous injection-induced fluorescent protein td Tomato expression in hepatocytes in vivo. (24)

4.1.3. Strategies for CRISPR/Cas9 based on proteins

A temporary, direct route for the introduction of the CRISPR/Cas9 system is provided by the administration of the Cas9 protein complex with sgRNA (together, the Cas9-RNP) using synthetic delivery vehicles. To effectively distribute the Cas9 protein within cells, David Liu's group combined spCas9 with supercharged GFP (-30GFP) to facilitate Lipofectamine® complexation. In vitro, this technique produced ∼50% NHEJ editing efficiency. The authors also postulated that encapsulation using the cationic lipid Lipofectamine® transfection reagent (RNAiMAX) would be made easier by the anionic charge of sgRNA. In the inner ear of transgenic Atoh1 (Atonal BHLH Transcription Factor 1)-GFP mice, a reporter model that expresses GFP fluorescence under the direction of the Atoh1 gene, complexes consisting of Cas9 protein, sgRNA, and RNAiMax, or RNAiMax alone, were injected. Because of its limited area and well-defined inner ear type, the inner ear was the goal. The mice's ears showed a 13% decrease in fluorescence ten days after injection. This work shed light on the idea of encapsulation and electrostatic assembly for Cas9-RNP delivery, even if it was mostly proof-of-concept. PEI polymers have also been utilized in vivo to help induce endosomal escape through Cas9 protein delivery, either alone or in conjunction with liposomes. A polymeric core shell nanoparticle coated with PEI on a DNA nanoclew containing a Cas9-sgRNA complex was described by Sun et al. Mice carrying U2OS-EGFP tumors were used to demonstrate in vivo administration and 25% EGFP disruption. The efficacy of editing was found to be better when there was partial complementarity between the sgRNA guide sequences and the DNA Nano clew. This implies that modifying the DNA nanoclew could allow for the incorporation of different sgRNAs for multiplexed editing. Recent research from Rotello's group showed that the Cas9 protein may be directly delivered into the cytosol while containing an oligo glutamic acid tag (E-tag, also known as Cas9En) at the N-terminus, which is an indication of a localized negative charge. This Cas9En-RNP coassembled with positively charged, arginine-functionalized gold nanoparticles (Arg-AuNPs) in combination with its sgRNA component to form a single delivery vector. Using an attached NLS, these supramolecular delivery vehicles allowed Cas9 to be transported directly to the cytosol and accumulate nuclearly. In almost 90% of cells, delivery was achieved through effective gene editing in the PTEN (30%) and AAVS1 (29%) gene loci. Although the in vitro outcomes of this study were encouraging, it is yet unclear whether this delivery vector is systemically applicable. CRISPR/Cas9 HDR repair vectors aimed at fixing the DMD-associated CXCR4 gene were integrated into a supramolecular delivery system in a recent study by Lee et al. This platform was made up of a 15 nm gold core that was adorned with thiolated DNA that could complementarity-hold a ssODN template. After the Cas9-RNP was electrostatically complexed inside the vehicle, the endosomal disruptive polymer PAsp (DET), where "DET" stands for

diethylenetriamine, was added to the complex. Using a local injection into muscle tissue, this delivery vector—dubbed CRISPR-Gold—achieved a 5.4% HDR efficiency and significantly improved the phenotypic agility of animal models in four-limb hanging tests. This breakthrough could transform the way that certain hereditary illnesses are treated, as local injections can help patients without requiring the use of viruses in these situations. More recently, mouse models of fragile X syndrome (FXS), which displayed prominent and unpredictable behaviors associated with this condition, were given cerebral injections using the CRISPR-Gold technique. Two weeks after the Grm5 gene and its related protein metabotropic glutamate receptor 5 (mGluR5, linked to the pathogenesis of FXS) were knocked down using CRISPR/Cas9, treated rats showed normalized behaviors and significantly reduced illness symptoms. However, the requirement for efficient endosomal escape is one factor to take into account with this approach. This poses a problem for many modern delivery systems and frequently reduces the effectiveness of delivery. Reduced off-target effects, almost no off-target mutagenesis, and a comparatively low immune response are provided by the transitory introduction of Cas9-RNP.Additionally, there is no chance of diminished effectiveness from the possible degradation of free sgRNA because the Cas9-RNP is pre-formed. It is difficult to deliver proteins into cells; nonetheless, endosomal entrapment is a problem for protein delivery methods because the majority of therapeutic proteins need to be located in the cytosol or, in the case of Cas9, in the nucleus. Furthermore, there are difficulties with translatability when using the Cas9-RNP; the Cas9 protein can be difficult to generate and loses its nuclease function quickly after isolation. (25)

4.2. Delivery of CRISPR-Cas9 using physical and non-viral

Target cells have been exposed to CRISPR-Cas9 by a variety of physical and non-viral delivery methods, including hydrodynamic injection, nanoparticles, and electroporation, during the system's early stages of deployment. The main benefit of non-viral vectors is safety, even if viral vectors—like the plasmid-based CRISPR/Cas9—deliver nucleic acids more effectively. Another benefit of non-viral delivery technologies is that transgenic DNA does not have a size restriction. Crucially, non-viral delivery technologies are more affordable and readily available than viral delivery system, which makes them a desirable option for applying the CRISPR-Cas9 system to human patients.

4.2.1. Electroporation

One popular method for introducing proteins and nucleic acids into mammalian cells is electroporation. During electroporation, the permeability of the cell membrane is momentarily raised, allowing proteins or nucleic acids to enter the cells.(26) All varieties of CRISPR-Cas9 systems, such as plasmid-based systems, Cas9/sgRNA RNPs, and mixtures of Cas9 mRNA and sgRNA, can be electroporated. (27)The integration of plasmid DNA into target cells is limited to about 0.01%, which is the drawback of electroporation. Furthermore, electroporation causes a considerable amount of cell death. Electroporation of plasmid-based CRISPR-Cas9 systems has been frequently used in gene editing investigations of vertebrate organogenesis to access zebrafish fin regeneration, axolotl regeneration in embryonic cells, chicken development, and mouse brain development. Furthermore, plasmid-based CRISPR-Cas9 has recently been delivered to cancer cells, CD4+ T cells, CD34+ stem cells, and embryonic stem cells using electroporation. Additionally, Cas9 mRNA and sgRNA have been electroporated into cells. To create a mouse model with altered genes, Cas9 mRNA, sgRNA, and donor DNA were, for instance, electroporated into mouse zygotes. Moreover, RNPs have been introduced into fibroblasts, embryonic stem cells, and CD4+ human T cells via electroporation.(28) In axolotl spinal cord cells, electroporation of RNPs was found to produce more gene-editing efficiency than electroporation of a plasmid-based CRISPR-Cas system. Liang et al. also conducted a comparison of RNPs, Cas9 mRNA/sgRNA, and the plasmid-based CRISPR-Cas9 system. When RNPs are electroporated into specific target cells, the effectiveness of gene editing is better than when equivalent plasmid-based CRISPRR-Cas9 or Cas9 mRNA/sgRNA are electroporated. For example, electroporation of RNPs resulted in 87% and 94% editing efficiency in Jurkat T cells and induced pluripotent stem cells, respectively. In contrast, the efficiency of electroporation of the Cas9 mRNA/sgRNA and the plasmid-based CRISPR-Cas9 system is lower in Jurkat T cells (63% and 42%, respectively) and induced pluripotent stem cells (20% and 32%, respectively). (29)

4.2.2. Microinjection

Using a glass micropipette, foreign compounds are directly injected into living cells at a microscopic level. Microinjection, a straightforward mechanical process, is now widely used in laboratories to introduce foreign protein or DNA into individual cells. The CRISPR-Cas9 system was directly injected using microinjection into embryonic cells or other highly reproducible and specific cell types.(30) The most straightforward way to edit genes is to inject plasmids encoding Cas9 and sgRNA into the pronucleus or nucleus. For instance, Mashiko showed that a quick and easy way to get a knockout mouse model in less than a month is to microinject a plasmid encoding Cas9 and sgRNA into the pronucleus of mouse zygotes. Similar to this, CRISPR-Cas9 microinjection was utilized to modify genes in the cells of worms, Aedes aegypti, zebrafish, rabbits, and Ciona intestinalis. Microinjection has been used to assess the effectiveness of various plasmid-based CRISPR/Cas9 methods for gene editing that target the same gene due to its simplicity and

accuracy.(23) Circular plasmid injection, however, might result in unintended side effects if the plasmid merges with the host chromosomes. Cas9 mRNA and sgRNA microinjection could prevent this. For example, Horii et al. demonstrated that the effectiveness of Cas9 mRNA/sgRNA microinjection into the pronucleus is better than that of Cas9 mRNA/sgRNA injection into the cytoplasm or Cas9 mRNA/sgRNA injection into the matching plasmid carrying Cas9 and sgRNA. While these findings showed that microinjection is a physically effective way to distribute Cas9 mRNA/sgRNA or plasmid-based CRISPR-Cas9, there are a number of drawbacks to microinjection. First off, because microinjection damages cells, it necessitates a great degree of physical dexterity and sophistication. Secondly, this technique is confined to a small number of cells; each injection can only target one cell. (31)

4.2.3. Hydrodynamic injection

Rapidly injecting a nucleic acid solution into mice through the tail vein in amounts equal to 8–10% of their body weight is known as hydrodynamic injection.(32) It has emerged as the most straightforward and effective way to provide nucleic acids to the liver since its discovery.(33) In order to create transient holes in endothelial cells' cell membranes and make it easier for nucleic acid to enter cells, hydrodynamic pressure is created by quickly administering a large volume of a nucleic acid solution. Numerous applications have made extensive use of hydrodynamic injection, including the transport of proteins, DNA, small interfering RNA (siRNA), and even cancer cells. Hydrodynamic injection was recently used to successfully administer the plasmid-based CRISPR-Cas9 system, which produced effective genetic repairs or mutations. In one work, a plasmid-based CRISPR-Cas9 system was used to correct the Fah mutation in a mouse model of the metabolic illness hereditary tyrosinemia by hydrodynamic injection into hepatocytes. In short, the pX330 backbone, which has cassettes for the Cas9 nuclease and sgRNA, is cloned to include a Fah-targeting sequence. After the plasmid and the updated Fah DNA template were hydrodynamically introduced into the animals, approximately 1/250 of the liver cells expressed the Fah protein. (34)The same group used hydrodynamic injection to deliver a pX330 system co-expressing a sgRNA sequence targeting PTEN into mice shortly after this investigation, and the results indicated that 2.6% of the liver genome's sequences were altered. Hydrodynamic injection of plasmid-based CRISPR-Cas9 systems allows for the quick development of liver cancer models and other disease models due to its ease of usage. Hydrodynamic injection works well for small animals but is not a suitable option for larger species. In addition to raising blood pressure, hydrodynamic injection causes transient cardiac failure, hepatic enlargement, and even animal mortality. Hydrodynamic injection is challenging to use in the clinic, in addition to having unknown effects on large animals. For instance, hepatotoxicity caused the clinical trial using hydrodynamic gene therapy to treat patients with cirrhosis to end prematurely.

4.2.4. Lipid Nanoparticles

One of the most popular methods for delivering nucleic acids is through lipid nanoparticles, and some of these have been put through clinical studies for RNA interference therapy. Lipid nanoparticles, which shield nucleic acids from nuclease and allow them to enter target cells by endocytosis or macro-pinocytosis, are often formed when negatively charged nucleic acids complex with positively charged lipids through electrostatic interactions. The use of lipid nanoparticles to introduce the CRISPR-Cas9 systems into various cells for medical purposes or to create knockout animal models has been investigated.(35) Without requiring any alterations, the same lipid nanoparticles that were produced for plasmid and siRNA can be directly used for the delivery of plasmid-based CRISPR Cas9 or the combination of Cas9 mRNA and sgRNA. To edit target genes in a variety of cell lines, including HEK293FT, U2OS, mouse ESCs, N2A, and A549, for instance, plasmid-based Cas9, Cas9 mRNA/sgRNA mixture, and even RNPs can be delivered using commercially available transfection lipids like Lipofectamine 2000, Lipofectamine 3000, and RNAiMAX. RNAiMAX shown a superior ability to distribute RNPs to cells with reduced toxicity when compared to Lipofectamin 2000. While RNPs for in vitro gene editing can be directly delivered by commercially available lipids, it is generally accepted that lipid nanoparticles for Cas9-sgRNA RNPs should be modified due to the positively charged nature of Cas9 protein, which is comprised of the complexation of cationic lipids and RNPs. Compared to plasmid-based CRISPR-Cas9, RNPs often exhibit greater gene editing efficiency and less off-target consequences. As a result, a lot of work has gone into creating novel lipid nanoparticles for RNPs. Zuris et al. showed that the RNPs created from the modified Cas9 and sgRNA may be effectively delivered using conventional cationic lipids by fusing the negatively charged protein (-30) GFP to the Cas9 protein. After a single treatment, the highly effective delivery of RNPs can induce up to 80% genome editing in cultured human cells. Additionally, 20% of the hair cells' genomes can be altered by delivering the modified Cas9/sgRNA RNPs into the mouse inner ear in vivo.(36)A number of bio-reducible lipids were created using a similar method to create nanoparticles containing changed RNPs for delivery into the mouse brain. To liberate the encapsulated RNPs, these bio-reducible lipids encourage endosomal release and the breakdown of the nanoparticles in the cytosol. In cultivated human HEK cells, lipid nanoparticles have a gene-editing effectiveness of about 70%. Additionally, the scientists showed that RNPs can be effectively delivered to the mouse brain using lipid nanoparticles for in vivo gene editing.

4.2.5. Polymer nanoparticles

Polymer nanoparticles are widely utilized for delivering several kinds of nucleic acids, such as oligonucleotides, RNA, and plasmid DNA. A cationic polymer called bPEI was covalently conjugated to the Cas9 protein in a recent work. The Cas9 protein was then complexed with sgRNA to form a CRISPR nanoparticle. When it comes to inducing DSB in its target gene, the polymer conjugated Cas9 keeps up its nuclease activity. Methicillin-resistant Staphylococcus aureus (MRSA) was successfully exposed to the CRISPR system via the polymer-based nanoparticle, which then successfully modified the target genome. Additionally, our approach outperformed unmodified Cas9/sgRNA complexed with traditional lipids in terms of editing efficacy. (37)

4.2.6. Gold nanoparticles

An innovative way to deliver RNPs is through gold nanoparticles. The Cas9 protein, which has been designed with a glutamate peptide tag and sgRNA, is used to co-assemble gold nanoparticles. In a range of cell types, this nanoparticle-mediated delivery approach achieves more than 90% transport efficiency and 30% gene editing efficiency. This may highlight the system's exceptional delivery efficiency. The modified Cas9 protein and sgRNA are delivered by nanoparticles via a cholesterol-dependent membrane fusion process that is different from cellular endocytosis. This technique offers a brand-new in vitro platform for temporary gene editing. Whether this system functions in human primary cells, such lymphoma cells, is still unknown to researchers. (38)

5. Challenges and Advancements in Crispr-Cas9 Delivery:(39)

5.1. Challenges

- **Size and Stability:** Due of their relative size, the gRNA and Cas9 protein have difficulty passively penetrating the cell membrane. They are also prone to being broken down by the body's enzymes. (40)
- **Targeting Specificity:** It's still difficult to deliver CRISPR components to particular cell types without inadvertently creating off-target consequences. (40)
- **Immunological reaction:** One popular mode of administration, viral vectors, may elicit an immunological reaction, which restricts the range of applications for therapeutic use. **Genotoxicity:** Cas9 off-target cleavage events have the potential to cause genotoxicity as well as unwanted mutations. (40)

5.1.1. Advances

- **Non-Viral Vectors:** To transfer CRISPR components, scientists are creating non-viral vectors such as liposomes, nanoparticles, and polymer complexes. When compared to viral vectors, these vectors have better safety profiles and can be designed to target particular cell types**.**
- **Tinier Cas Proteins**: To lessen delivery issues related to Cas9 size, smaller Cas variants such as Cas12a and Caš (phi) are being investigated.
- **Delivery Techniques:** For the targeted delivery of CRISPR components, novel delivery techniques including electroporation and ultrasound are being researched.
- **Guide RNA Optimization**: Stabilization and reduction of off-target effects can be achieved by chemically modifying gRNA.
- **Delivery Carriers:** For better biocompatibility and targeted delivery, red blood cells and modified exosomes are being investigated as viable delivery carriers. (41)

5.1.2. Examples of Advancements

- **Lipid nanoparticles**: In animal studies, lipid nanoparticles have demonstrated potential in transporting CRISPR components to the brain, liver, and lungs.
- **Base editing with CRISPR-Cas9:** This method reduces the possibility of off-target consequences by precisely modifying single nucleotides without creating double-strand breaks.
- **The Delivery of CRISPR-Cas9 in the Future:**

The delivery of CRISPR-Cas9 is a fast-developing field. The goal of ongoing research is to create novel delivery methods that are safer, more precise, and more efficient. Personalized medicine techniques and the full therapeutic potential of CRISPR-Cas9 will be made possible only by advancements in these domains. (42)

6. Applications of Crispr-Cas9 to Create Novel Treatments

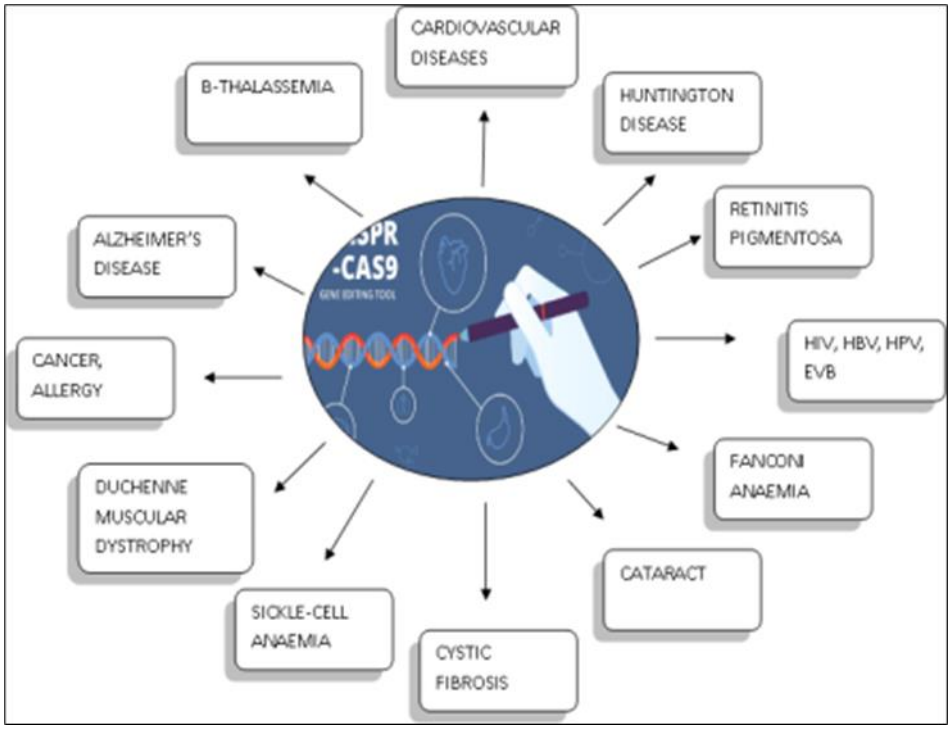

Figure 1 Gene Adjustment in Monogenic Illnesses

- **Cystic Fibrosis:** This condition is brought on by mutations in the CFTR gene. CRISPR exhibits potential in rectifying these mutations and reinstating the CFTR protein's functionality.(42)
- **Sickle Cell Disease:** Sickle cell anemia is caused by a single nucleotide alteration in the HBB gene. A treatment might be possible if this mutation is precisely corrected using CRISPR.(43)
- **Beta-Thalassemia:** This condition is likewise brought on by mutations in the HBB gene. Gene editing using CRISPR may be able to fix these mutations and reduce symptoms.(44)

6.1. Changing the Genes That Cause Disease

- **Huntington's disease:** An enlarged CAG repeat in the HTT gene causes this neurological condition. To stop the progression of the disease, this gene could be silenced or disrupted using CRISPR. (45)
- **Duchenne Muscular Dystrophy (DMD):** This condition is caused by mutations in the DMD gene. To repair these mutations and add therapeutic exons to restore muscle function, CRISPR presents an option.(46)
- **HIV:** By altering the CCR5 gene, which acts as an HIV entrance site, CRISPR may be utilized to make cells resistant to infection.(47)

6.2. Cancer Immunotherapy

T-cells, the body's immunological troops, can be made more capable of identifying and destroying cancer cells by the application of CRISPR. (48)

Using CRISPR editing to modify T-cell receptors or chimeric antigen receptors (CARs) can improve their capacity to recognize and eradicate cancer cells.

6.3. Gene editing for Regenerative Medicine

CRISPR allows for the modification of stem cells, which are the body's means of repair, in order to treat a number of illnesses.

For instance, heart disease, diabetes, and neurodegenerative diseases may be treated by modifying stem cells to develop into healthy tissues.(49)

6.4. Gene Treatment for Blood Conditions

Using CRISPR to fix mutations in blood stem cells, blood diseases such as sickle cell anemia and beta-thalassemia may be treated.(50)

7. Challenges and Ethical Issues with Crispr-Cas9 Gene Editing

Although a potent tool, CRISPR-Cas9 poses several obstacles and ethical issues that must be resolved prior to a broad clinical implementation. A summary of these main issues is provided below:

7.1. Challenges

- **Off-Target Effects:** Obtaining accurate targeting is one of the main challenges. Unintentional genome cleavage by Cas9 can occasionally result in dangerous alterations.
- **Delivery Techniques**: It's still difficult to deliver the large Cas9 protein and guide RNA (gRNA) effectively and safely to target cells.
- **Efficiency:** Although CRISPR has a high editing efficiency, more advancements are required to guarantee dependable and consistent editing results.
- **Unintended Immune Response:** The efficacy of CRISPR therapy may be jeopardized if foreign genetic material is introduced because it may cause an immunological reaction. (51)

7.2. Ethical Concerns

- **Germline Editing:** Changing the germline can have long-term and unexpected ramifications, as it can pass genetic modifications on to subsequent generations.
- **Eugenics:** There are ethical concerns around the possible misuse of CRISPR to create "designer babies" with desired features.
- **Equity and Access:** Those who are less fortunate may find it more difficult to receive CRISPR medicines because of its high cost.
- **Informed permission**: Getting informed permission from patients requires making sure they are completely aware of the possible hazards and advantages of CRISPR therapy. (7)

8. Guidelines and Regulatory framework

The concerns that prompted the formation of the Second International Summit on Human Genome Editing were brought to light by He Jiankui's presentation of the first children born with germline editing. To build a framework that would help scientists, clinicians, and regulators alike when evaluating applications of human genome editing, the U.S. National Academies of Sciences, Engineering, and Medicine and the Royal Society of the United Kingdom organized the inaugural summit as part of an international project. The committee released a thorough guide on human genome editing in 2017, coming to the conclusion that the regulatory frameworks currently in place for human gene therapy were adequate to oversee somatic genome editing research as long as it was focused on curing illnesses and disabilities and assessed the safety and effectiveness of people in clinical trials, and before concluding the trial phase, they asked the general public for feedback on the treatment.(52) Regarding human germline editing, the committee came to the conclusion that while research might be allowed in certain situations to treat severe illnesses and disabilities, there would need to be stringent oversight to ensure that the technology could not be used for other purposes, as well as ongoing public involvement in the process before proceeding. They further pointed out that financing for research involving the deliberate alteration of human embryos is currently prohibited in the US by the FDA.(52) The Dickey-Wicker Amendment, which is annexed to the appropriations bills for the Departments of Since 1996, the Department of Health and Human Services (DHHS) has been barred from utilizing approved funding for research involving human embryos. This includes the National Institutes of Health. As previously noted, the FDA continues to prohibit financing for studies involving human embryos. The House of Representatives Appropriations Committee most recently passed a rider to a 2020 bill that prohibits the FDA from approving any clinical trials that result in heritable modifications in June 2019. FDA regulation and DHHS approval are required for somatic gene editing medicines. The FDA published new guidelines for clinical trials and the steps involved in producing these treatments at the beginning of 2020.(52)By assembling an international panel, the World Health Organization is also attempting to create supervision and governance procedures of professionals. The advisory council effectively recommended a moratorium akin to those previously advised when it released a statement in July 2019 denouncing the clinical application of germline genome editing until its ramifications have been carefully explored. Further, this committee has recommended the creation of a global framework based on the guiding principles of social justice, transparency, inclusivity, fairness, and responsible scientific stewardship. The framework should be developed in cooperation with the greatest number of stakeholders and be scalable from local to international

governments as well as work in environments where scientific practice is subject to both stringent and lax regulations.(53) In an effort to increase public transparency about human genome editing research, further international initiatives have included the establishment of a global registry to monitor studies in both somatic and germline contexts procedure for studying human germline editing.

9. Conclusion

This review has provided a comprehensive assessment of CRISPR-Cas9 gene editing therapies, examining both their immense potential and the significant hurdles that lie ahead. While CRISPR-Cas9 offers a powerful tool for addressing genetic disorders and revolutionizing medicine, its therapeutic application requires careful consideration of its efficacy, safety, and ethical implications. Addressing challenges like unintended edits, delivery methods, and immune responses will be crucial for its successful translation to the clinic. Furthermore, robust ethical frameworks and regulations are necessary to guide research and development, ensuring responsible use of this powerful technology. By fostering informed discussions and prioritizing safety, we can harness the potential of CRISPR-Cas9 to deliver on its promise of a new era in healthcare.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors do not have any conflict of interest.

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