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REVIEW ARTICLE

The Advantages of Base and Prime Editing over Traditional CRISPR in the Treatment of Monogenic Diseases

Jessica Ferrie

Harvard-Westlake School, 3700 Coldwater Canyon Avenue, Studio City, CA 91604; jessicacferrie@gmail.com
Mentor: Sarah Sandler

ABSTRACT: CRISPR-Cas gene editing tools have revolutionized the field of biotechnology. Originally part of a bacterial immune system, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated proteins (Cas) can cleave specific DNA sequences that match the RNA sequence coded into the Cas protein. CRISPR has great potential for curing genetic diseases and cancers. However, many challenges must be overcome, including the risk of off-target cuts and their resulting consequences, on-target effects, adverse immune system reactions, the need for a double-stranded DNA break (DSB), and the need for a donor template to carry out homology-directed repair. Alternatively, base editing and prime editing are technologies that can edit genes without a DSB and donor template. There is also a reduced risk of off-target editing, which mitigates a significant safety concern. A key application of CRISPR and other gene editing technologies is curing monogenic diseases, which arise from mutations in a single gene. This makes them simpler to treat than other types of genetic diseases. Here, we present the principles of CRISPR, base editing, and prime editing, and discuss how they can be applied in a clinical setting to treat or cure monogenic diseases.

KEYWORDS: Cellular and molecular biology; Genetics; Gene editing; CRISPR; Base editing; Prime editing.

Introduction

Gene editing as a concept has been around since the mid-20th century. Initially, efforts toward curing genetic diseases involved inserting a healthy version of the gene into cells rather than correcting the mutation itself. This approach was only effective in certain limited cases.[1] To correct genetic mutations, scientists would have to cause a double-stranded DNA cleavage at a specific site in the genome, and provide the cell with a template strand that could be followed to repair the DNA, introducing the desired edit. However, having the double-stranded break occur at precisely the right location has proved to be a challenge, and the chance of off-target cuts creates a barrier to developing safe and effective gene therapies for human patients.

The first two prevalent gene editing technologies developed were zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). ZFNs use the bacterial restriction enzyme FokI, which can bind to and cut DNA. With ZFN editing, a pair of three-finger ZFNs makes a double-stranded cleavage in the DNA. With a donor DNA template, the cell can then perform homology-directed repair, replacing the mutated gene with a healthy version of that gene.[2] TALENs work similarly to ZFNs, binding to and cutting DNA using FokI. However, while ZFNs recognize DNA in groups of three, each TALEN domain recognizes a single DNA base.[3] Although TALENs are easier to design than ZFNs, TALENs are much larger than ZFNs, making them difficult to deliver into cells.[4] CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas (CRISPR-associated) proteins are widely used as an alternative to ZFNs and TALENs. CRISPR/Cas9 is more appealing due to its ease of program and multiplex and its increased efficiency. Jennifer Doudna (University of California, Berkeley, CA, USA) and Emmanuelle Charpentier (Max Planck Unit for the Science of Pathogens, Berlin, Germany) were awarded the Nobel Prize for Chemistry in 2020 for developing CRISPR/Cas9 gene editing technology.[5]

CRISPR/Cas9-based Gene Editing Technologies:

CRISPR/Cas-based technologies have evolved into new methods of gene editing, such as base editing and prime editing. Three important gene editing technologies used today are traditional CRISPR/Cas9, base editing, and prime editing, as seen in Figures 1a, 1b, and 1c.

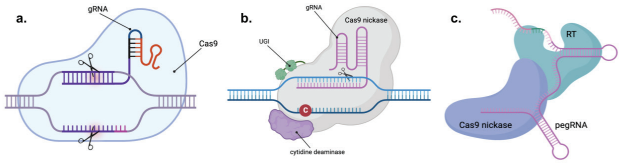

Figure 1: Gene Editing Technologies a. Traditional CRISPR consists of a Cas9 enzyme that makes a double-stranded break at the target site based on the gRNA. The cell then repairs the DNA either through non-homologous end-joining or homology-directed repair using a donor template. **b.** The base editor uses a modified version of the Cas9 protein - Cas9 nickase (Cas9n) - so the DNA is nicked on only one strand. The Cas9n nicks the DNA at the indicated site on the gRNA, and the deaminase enzyme performs the base conversion. The cytosine base editor has an additional component - a UGI (uracil DNA glycosylase inhibitor), which inhibits the base excision repair pathway in which the cell removes damaged or foreign bases, increasing the efficiency of the base edit. **c.** The prime editor also employs the Cas9n protein, which forms a complex with a pegRNA and a reverse transcriptase (RT) enzyme. The pegRNA guides the prime editor to the target DNA region, the Cas9n nicks one strand of the DNA, and the RT transcribes the edit on the pegRNA onto the DNA. This figure was created using biorender.com.

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CRISPR originated in bacteria as a natural part of their immune system. Bacteriophages are viruses that infect bacteria and use bacteria to replicate themselves. They attack bacteria by inserting their DNA into them.[6] If the bacterium successfully fights off the infection, it will insert part of the viral DNA into its own genome next to a CRISPR sequence. As seen in **Figure 1a**, the Cas proteins then form a complex with a guide RNA transcribed from the viral DNA, and patrol the bacterium for a DNA sequence complementary to the guide RNA. If such a sequence is found, the RNA-Cas complex will bind to the DNA and cleave it, disposing of the infection. This mechanism can be modified to edit genes. CRISPR/Cas9 gene editing requires three components - the Cas9 protein, a single guide RNA, and the presence of a protospacer adjacent motif (PAM) in the target sequence.[7] Cas9 is an endonuclease enzyme that recognizes the PAM sequence (NGG), binds to the DNA, and cleaves adjacent to it. Single guide RNA is an RNA sequence that tells the Cas9 where to cut DNA and consists of a guiding region (crRNA), which is 17-24 bases long and complementary to the target DNA region, and a scaffold region (tracrRNA), which forms a hairpin loop that recruits Cas9. The PAM sequence is found immediately after the target sequence, letting the Cas protein know where to bind. For Cas9, the most commonly used enzyme for gene editing, this sequence is NGG, where N is any DNA base (A, C, T, G). Following the double-stranded break, the DNA is repaired using one of two methods: homology-directed repair or non-homologous end joining. Non-homologous end joining uses the cell's natural repair mechanisms to insert, delete, or change bases, creating mutations that would prevent the gene from functioning correctly. Homology-directed repair is when the DNA is repaired using a donor DNA template. A donor template consists of an insert between sequences of DNA that match the target region. The matching DNA on either side of the insert causes the strands to bind together with the existing DNA, allowing the insert to be copied.

A key difference between base editing, prime editing, and traditional CRISPR is that base and prime editing do not require a double-stranded DNA break or a donor DNA template. Base editing was first developed by Anzalone *et al.* in 2016.[8] It can chemically convert one DNA base into another. Two base editing mechanisms exist: the adenine base editor (ABE) and the cytosine base editor (CBE). The ABE can convert adenine to guanine or vice versa, and the CBE can convert cytosine to thymine or vice versa. This is useful as 32% of monogenic diseases are caused by C/G to T/G variants, which base editing can fix.[9] The components of a base editor are a Cas9 nickase (Cas9n)-guide RNA (gRNA) complex linked to a deaminase enzyme, either cytidine or adenosine deaminase (depending on the desired base conversion) (**Figure 1b**). Cas9n is a modified version of Cas9 that cuts only one, not both, strands of DNA. With the CBE, there is an optional component, a uracil glycosylase inhibitor (UGI), which increases the efficiency of the edit. When the base editor enters a cell, the Cas9n-gRNA complex and linked deaminase enzyme will bind to DNA, dictated by the guide RNA. The Cas9n will nick the top strand, and the deaminase enzyme will carry out the base conversion. The cell will then repair the DNA, finalizing the edit.[10] Since base editing does not require a DSB, multiple base edits can co-occur. However, a significant limitation is that base editing can only perform transition mutations instead of transversion mutations. This means it can only convert a purine into a different purine and a pyrimidine into another pyrimidine rather than converting a purine to a pyrimidine or vice versa.[11]

Prime editing was also developed by Anzalone *et al.*[12] Based on CRISPR technology, it can insert and delete bases and perform any base conversion. The prime editor consists of a modified Cas9n-prime editing guide RNA (pegRNA) complex linked to a reverse transcriptase (RT) enzyme. The pegRNA consists of a spacer sequence, a primer binding site (PBS), a reverse transcriptase template (RTT), and a region that binds to the Cas9n and RT (**Figure 1c**). With prime editing, the prime editor binds to DNA based on the pegRNA spacer sequence. The Cas9n performs a single-stranded nick on the DNA after recognizing the PAM sequence, and the RT uses the pegRNA's RTT to transcribe the edit. The cell repairs the DNA by removing the duplicate sections, either by a 5' flap or a 3' flap. The prime edit only works if the 3' flap occurs; if the 5' flap occurs, the edit will be lost.

Monogenic Diseases:

Many genetic disorders stem from genetic mutations when one or more bases in the genome are inserted, deleted, or changed. This can disrupt gene function, creating a dysfunctional protein that could have detrimental effects on the body. Monogenic diseases are a subset of genetic disorders where the genetic disorder results from a mutation in a single gene. Over 5,000 known monogenic diseases affect more than 250 million people worldwide.[11] This paper discusses three monogenic diseases: cystic fibrosis, Tay-Sachs, and Duchenne's muscular dystrophy, and studies that show potential for treating them through gene editing.

Cystic fibrosis (CF) is a progressive monogenic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leading to a dysfunctional CFTR protein. Almost 40,000 people in the United States live with CF, and it affects around 105,000 people worldwide.[13] The CFTR protein affects the body's cells, tissues, mucus, and sweat-producing glands. CFTR moves chloride, which attracts water, to the cell's surface, regulating mucus consistency. Normal mucus is slippery and protects the lining of organs and tissues, while people with CF make thick, sticky mucus that builds up in organs, causing blockage, damage, and infection.[14] Cystic fibrosis affects many organs in the body, including the lungs, pancreas, and liver. In the lungs, mucus clogs airways and traps germs, causing infection, inflammation, and respiratory failure. In the pancreas, mucus prevents the release of digestive enzymes, causing poor nutrient absorption, malnutrition, and inhibited growth. Mucus also blocks the bile duct, leading to liver disease. Current treatments for CF include airway clearance to help loosen and remove thick mucus buildup in the lungs; inhaled medicines to open airways, thin mucus and fight lung infections; pancreatic enzyme supplements to aid in the absorption of nutrients; and a personalized fitness plan to improve energy, lung function, and general health.[13]

CFTR modulators are also used to target defects in the CFTR protein, although it is only effective for specific mutations in the CFTR gene. Advancements in CF care have improved patients' duration and quality of life, and now, over half of CF patients are adults. Despite this, there is no cure. Patients are more likely to develop other health conditions, and often die early.[15]

Tay-Sachs disease is a very rare but very deadly disease. Its most common and deadliest form is the infantile form; people who suffer from it typically live for only a few years.[16] Tay-Sachs disease is caused by mutations in the HEXA gene, which codes for part of the beta-hexosaminidase A enzyme. This enzyme is located in lysosomes, which break down and recycle toxic substances in cells. The enzyme is required to break down the fatty substance GM2 ganglioside in the cell membrane. Without this enzyme, lipids will accumulate to toxic levels in the cell, causing nerve cell and central nervous system damage.[17] The symptoms of Tay-Sachs start to appear around six months, when the infant will stop meeting developmental milestones and lose basic motor skills, including the ability to crawl, sit, turn over, etc. Symptoms include seizures, slow development, cherry-red spots in the eyes, vision and hearing loss, difficulty swallowing, increased startle reflex, and muscle weakness resulting in paralysis.[18] Since there is no cure for Tay-Sachs, treatments focus on minimizing the child's suffering and maximizing comfort through anti-seizure medication, respiratory care, special nutrition and hydration techniques, and physical, occupational, and speech therapies.

Duchenne's muscular dystrophy (DMD) is a monogenic disease classified as one of four dystrophinopathies, which are conditions that cause muscle weakness and degeneration due to an absence or dysfunction of dystrophin.[19] DMD is the most severe and deadly type of dystrophinopathy, and the incidence rate is between 1 in 3,500-5,000 males worldwide. Dystrophin is a protein coded for by the encoding dystrophin genes and is needed to keep muscle cells intact. It absorbs shock when muscles contract; without it, the muscle cells become damaged, and fibrosis occurs, which replaces the muscle with scar tissue and fat.[20] This leads to muscle tissue loss, and they eventually cease to function. The encoding dystrophin gene is inherited on the X chromosome, so DMD occurs much more often in males than females.[21] DMD is caused by frameshifting or nonsense mutations in the dystrophin gene, creating dystrophin that is unstable or doesn't function.[22] The symptoms of DMD include a delayed ability to sit, stand, walk, and speak; and difficulty jumping, running, and walking. In addition to affecting skeletal muscle, DMD also affects the heart and respiratory muscles. The progression of the disease begins around the age of 2-3 when the patient starts exhibiting symptoms. Around the age of 10-12, the patient will require a wheelchair, and around the age of 20, the patient will require assisted ventilation. Patients typically die between the ages of 20 and 40 due to cardiac or respiratory failure.[22] Although DMD does not yet have a cure, the FDA has approved various medications to mitigate its effects. Emflaza is an anti-inflammatory and immunosuppressive drug that slows the loss of muscle strength and function, preserves cardiac and respiratory function, and reduces scoliosis. Other medications such as Vyondys 53, Exondys 51, Viltepso, and Amondys 45 skip specific exons in the encoding dystrophin gene and are mutation specific, meaning that they are effective only with certain DMD mutations.[23]

These monogenic diseases, among others, have been widely researched, and various studies have demonstrated potential treatment pathways utilizing Cas-based gene editing technologies. Monogenic diseases can be caused by a range of mutations in the same gene, so different treatments must be developed for different patients and variants of the disease. A cystic fibrosis study by Schwank *et al*. utilized CRISPR/Cas9 and homologous recombination to correct the mutated CFTR gene in cultured intestinal stem cells from humans and mice. Following treatment, the corrected CFTR gene was expressed and functional, showing promise for gene correction of CFTR in adult stem cells.[24] In addition, a study by Krishnamurthy *et al*. utilized an adenine base editor delivered by an RNP to correct three different CFTR mutations. Treatment resulted in the correction of the mutations ranging from 38-82% efficiency and functional correction and expression of the CFTR gene. Off-target edits and indels were minimal.[25] This study demonstrates the potential of base editing as a viable treatment method. Gene therapies for Tay-Sachs aim to repair the Hex enzyme by correcting the HEXA gene and reducing the buildup of GM2 ganglioside. In a study by Ou *et al*., researchers utilized a CRISPR system delivered by AAV to introduce a modified Hex gene into neonatal mice with Sandhoff disease. Four months after treatment, GM2 gangliosides were reduced to normal levels in multiple tissues except for the brain, coordination and motor skills were improved, and cellular vacuolation rates decreased in the brain and liver. This study demonstrates the potential for an *in vivo* treatment for TS and Sandhoff diseases since both originate from Hex-encoding gene mutations (HEXA and HEXB, respectively).[26] Leal *et al*. utilized a Cas9 nickase delivered by liposomes to treat Tay-Sachs and Sandhoff fibroblasts. Results were mostly positive - donor HEXA integration was successful and produced significantly increased enzyme activity during a 7 and 15-day period. Moreover, oxidative stress was positively impacted by the treatment. However, lysosomal mass, which is an indicator of GM2 ganglioside buildup, was not improved by treatment.[27] In a study about Duchenne muscular dystrophy conducted by Iyombe-Engembe *et al*., researchers used CRISPR-induced deletion to target exon deletions, causing a frameshift mutation in the DMD gene. They deleted large portions of the targeted exons, enabling them to fuse and repair the DMD gene at a rate of 62%. This method demonstrates the promise of CRISPR-induced deletion as an alternative method to exon skipping or deletion of entire exons in treating DMD mutations.[28] A study conducted by Lattanzi *et al*. utilized CRISPR to correct the exon two duplication in DMD. They carried out the deletion in myogenic cells from DMD patients, restoring dystrophin expression. This demonstrates a potential cure for patients with exon 2 duplication DMD.[29]

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Discussion

Gene editing technology has the potential to cure monogenic diseases. However, significant challenges must be overcome before developing safe and effective gene therapies. First, the technologies' limitations must be addressed, such as accuracy and precision.

CRISPR, base editing, and prime editing each have their own advantages and drawbacks. CRISPR has great breadth and scope regarding the types of gene edits it can execute. It is an established technology used in various situations and has successful results.[30] Of the three technologies, the CRISPR/Cas9 complex is the easiest to deliver into cells since it is the smallest. Currently, the prime editor complex is too large to perform RNP electroporation into cells; its size presents a barrier to its widespread use. CRISPR requires a double-stranded DNA break and a donor template for homology-directed repair, which introduces safety risks and dramatically increases the chance of errors. CRISPR and prime editing can perform all types of base substitution, insertion, and deletion, making them incredibly versatile. An advantage of prime editing is that the PAM requirement for prime editing is less strict than CRISPR.[31] Alternatively, base editing can only perform base substitution edits between two purines or two pyrimidines, presenting a significant obstacle in scope and the types of mutations it can target. It is also inefficient because base editors can only convert one base at a time. The biggest concern with gene editing is the risk of off-target effects. Base editing and prime editing have little to no off-target effects, while CRISPR has significant off-target effects because it requires a DSB and a donor DNA template.[32] Another concern is on-target effects, which include translocations and unwanted deletions and insertions at the target site (indels).[33] The on- and off-target effects of base editing and prime editing have not been as extensively studied as CRISPR due to the novelty of these technologies, so further research is necessary to understand the risks and efficacy of these technologies fully.

In addition to issues with gene editing technologies, some obstacles occur when the technology is applied in a real-life setting. The gene editor can be delivered into cells either *ex-vivo* or *in-vivo*. *Ex-vivo* editing is when cells are extracted from the patient, edited, and returned to the patient. Examples of *ex-vivo* delivery methods include electroporation of ribonucleoprotein complexes (RNPs). *In-vivo* editing is when the gene editing tools are inserted directly into the patient. *In-vivo* delivery methods include lipid-based nanoparticles and viral vectors. Each delivery method has its own set of benefits and drawbacks.[34]

RNP complexes are an *ex-vivo* delivery mechanism comprising a purified protein and guide RNA. They carry enzyme complexes and are typically delivered into a patient's stem cells via electroporation to treat conditions like blood disorders. The advantage of RNPs is a reduced risk of genomic integration in off-target sites, and there is a lower risk of off-target effects due to a lack of long-term gene expression since RNPs naturally degrade soon after delivery into cells. The disadvantages of RNPs are that they cannot enter cells independently, degrade if left unprotected, and develop immunogenicity *in vivo*. RNPs are useful for short-term gene expression, have limited potential for off-target effects, and do not use donor DNA.[35] They can target oocytes, stem cells, and T cells. RNPs are usually electroporated into cells. During electroporation, the cells are given an electric shock, which creates holes in the cell membrane. This allows the gene editing tools to enter the cell. However, electroporation can have a high cell death rate.[36]

Lipid-based nanoparticles and viral vectors are examples of *in-vivo* delivery methods. Lipid nanoparticles surround the molecules they are transporting, allowing them to be taken up by the cell. The benefits of lipid nanoparticles are that they are relatively cheap and easy to make, have low immunogenicity, and have no risk of genomic integration in off-target sites. However, they are toxic and have a limited range of tissue types they can target. Lipid nanoparticles range in size - between 50-500 nanometers - and can target cells in the liver. Viral vectors are viruses that have their DNA replaced with the desired gene and deliver the gene into cells. Adeno-associated viruses (AAVs) are the most commonly used vector for *in-vivo* delivery, although other vectors used in gene therapies include adenoviruses, retroviruses, and lentiviruses.[37] Viral vectors are efficient, can target a wide range of tissues, and are a clinically established delivery method. Their drawbacks include the risk of genomic integration in off-target sites, the possibility of off-target effects due to long-term expression, immunogenicity, and their limited size.[38] AAVs are around 20 nanometers in size, so they often cannot carry all of the required gene-editing components in one viral vector. It can carry the components for CRISPR-Cas9 gene editing but not the base and prime editors, which use more extensive versions of the CRISPR/Cas9 technology. Viral vectors are versatile in the types of tissue they can target, including the liver, eyes, brain, lungs, and muscle tissue.[39]

Despite the challenges and limitations of gene editing technology, studies and clinical trials have been conducted involving gene editing and CRISPR. Base editing has been successfully used to treat genetic diseases and cancer patients. For example, it was used in a CAR-T cell therapy to cure a child's leukemia when the patient failed to respond to both chemotherapy and a bone marrow transplant.[40] Verve Therapeutics started a clinical trial in 2022 testing their treatment VERVE-101, which uses an adenine base editor that targets and turns off the PCSK9 gene in the liver. This would reduce low-density lipoprotein cholesterol (LDL-C), which causes hypercholesterolemia.[41] In 2022, the FDA approved the first test of a treatment utilizing CRISPR/Cas9 that treated patients with sickle cell disease in a collaboration between the University of California, Berkeley, and UC San Francisco.[42] Vertex Pharmaceuticals and CRISPR Therapeutics developed a gene therapy for sickle cell disease and beta-thalassemia called exa-cel, which enables the expression of the fetal hemoglobin gene. The clinical trial has yielded promising results. Following one infusion of the treatment, 75 patients with sickle cell disease and beta-thalassemia had the worst of their symptoms alleviated. All but two beta-thalassemia patients stopped requiring blood transfusions.[43] This treatment was approved by the FDA in 2023, and its market name is Casgevy. The FDA also approved another treatment

for sickle cell disease: Lyfgenia, which was developed by Bluebird Bio.[44] These historic milestones mark a significant step toward implementing gene therapies for patients suffering from genetic disorders.

Ethics and Accessibility of Gene Editing:

Gene editing technologies have the power to change the world and positively impact many people. However, they raise important questions about the ethicality of when and to what extent they should be used.

When a person receives a genetic treatment, only their somatic cells have the gene edit. A way to permanently fix a genetic mutation across generations would be to edit an embryo so subsequent offspring develop from cells without the mutation. This is called germline editing and is highly controversial. There is a debate about the human status of an embryo and whether it should be considered a human with fundamental rights, including the right to informed consent, versus a collection of genetic material. This debate fuels the question of whether experimentation on human embryos should be legal. While the long-term effects of experimentation on embryos are unknown, it might provide important information that could lead to the development of regenerative treatments for disorders such as Parkinson's disease, spinal cord injury, cardiomyopathy, and more.[45] Additionally, germline editing may allow scientists to "optimize" a baby's genes, such as giving it high intelligence, good vision, muscle and height, or low risk of disease. However, this could be considered trying to create the "ideal human," which sparks eugenics concerns.

Another important ethical question involves whether gene editing should be used in non-therapeutic settings, such as genetically modifying crops, gene drives, and enhancing human features. Millions of people worldwide cannot obtain enough nutrients, so gene editing could be leveraged to find potential solutions. Gene editing has the power to make crops and animal products more nutritious. However, the possible harms of consuming genetically modified organisms (GMOs) must still be fully understood. A gene drive is where gene editing is used to increase the rate of genetic recombination in a population so that a specific gene can be quickly distributed to the whole population. This can be used to edit the genes of an entire population or perhaps a whole species.[45] Gene drives have been used in mosquitoes, fruit flies, plants, fungi, and mice.[46,47] Researchers at UCSD have bred the *Aedes aegypti* family of mosquitoes to express an antibody that protects them from the major strains of dengue. They are currently creating a gene drive that will kill a mosquito when it gets infected by a virus. Gene drives have the potential to fight deadly diseases, control invasive species, and save millions of lives per year. However, it is important to remember the dangers of such a powerful technology - irreversibly altering the genome of a species can have significant unintended effects, such as hurting the larger ecosystem. In response, researchers have developed safety measures in gene drives, such as built-in controls, external overrides, and more, to prevent disasters and counter or reverse them if needed.[45] In humans, the line between gene editing treatment for therapeutic purposes and treatment for enhancement purposes can be uncertain; "necessity" does not have a clear definition.

Accessibility is another significant challenge with gene editing. Gene therapy is expensive. Currently, the gene therapies that have been approved cost anywhere between 373,000 USD and 2.1 million per treatment. Even in high-income countries, this is an exorbitant price, and for low- and middle-income countries (LMICs) whose average annual income is less than or equal to USD 12,535, these therapies are entirely out of reach. The vast majority of people with monogenic diseases such as HIV and hemoglobinopathies (e.g., sickle cell disease and beta-thalassemia) live in LMICs. Still, almost all clinical trials for gene therapies targeting these diseases are done in high-income countries, with only a few trials being conducted in LMICs.[48] In 2017, the first gene therapy treatment, called voretigene neparvovec, was released in the United States to treat eyesight loss from inherited retinal dystrophy. It costs USD 425,000 per dose for one eye. A pediatric spinal muscular dystrophy treatment called onasemnogene abeparvovec costs USD 2.1 million per dose.[49] Kymyriah, a CAR-T cell therapy, costs USD 475,000. The average cost of one dose of gene therapy is around 30 times the United States' median household income, meaning many patients who require treatment cannot afford it. As gene therapies become more popular and their use becomes more frequent and widespread, production costs could decrease, although treatment would still be costly. These costs would have a greater effect on disadvantaged communities and ethnic minorities, who already, on average, are disproportionately affected by disease, have lower incomes, and less access to healthcare. This could exacerbate inequalities in medicine.[49]

■ Conclusion

Gene editing can potentially cure, rather than treat, the symptoms of monogenic diseases. Cystic fibrosis, Tay-Sachs disease, and Duchenne muscular dystrophy are examples of genetic disorders that can be solved with gene editing. Although, in principle, gene editing is a very elegant solution, in practice, there are significant barriers to developing effective and safe gene therapies. These limitations include on- and off-target effects, scope, and delivery issues.

Jennifer Doudna and Emmanuelle Charpentier developed CRISPR technology and won the Nobel Prize in 2020. CRISPR uses an enzyme found in bacteria to locate a target gene sequence, perform a double-stranded DNA break, and insert a desired gene edit. However, CRISPR has a high rate of off-target cuts, which can have detrimental effects on the patient. Base editing and prime editing are emerging technologies that offer a safer and more effective alternative to CRISPR. Base editing shows a lot of promise as it is low-risk and permanent. Since many genetic diseases, including sickle cell disease and Tay-Sachs, are due to base substitutions, base editing could cure a large portion of monogenic diseases. However, no technology is perfect, and there are still obstacles to overcome with base and prime editing. Although CRISPR's utility and contributions should not be overlooked, ultimately, base editing and prime editing are more appealing alternatives to

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traditional CRISPR because they do not require a DSB, have more flexibility for the PAM sequence, are more efficient, and current studies show that they have fewer on- and off-target effects.

Despite the issues with gene editing technologies and the difficulties with applying them in the real world, companies have developed gene therapies for genetic disorders. They are conducting clinical trials for the treatments, and a few have been approved. Gene editing has already begun to impact patients' lives positively. Victoria Gray is the first person with sickle cell disease to receive a treatment utilizing CRISPR gene editing. Now, her symptoms are gone, and her quality of life has improved drastically.[50] In addition, the first gene therapy using CRISPR/Cas9, called Casgevy, was approved by the FDA in 2023, treating patients with sickle cell disease.[44] Future advances in gene editing and more clinical trials will continue to pave the way toward curing genetic diseases and changing patients' lives for the better.

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Author

Jessica Ferrie is a sophomore at Harvard-Westlake School in Studio City, California. She is interested in molecular biology, biochemistry, and biotechnology and hopes to pursue STEM at the university level and as a career.

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