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The Methods, Benefits, and Dangers of Genetic Therapy

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Abstract: *Gene transfer as a potential cure for inherited disorders has been discussed by many human geneticists. It is now more likely than ever that this pipe dream will come true, and that gene therapy won't just be used to treat diseases caused by a single gene but will also have applications in many areas of medicine thanks to the remarkable advancements made in recombinant DNA technology and cell biology in recent years. In treating a variety of illnesses, genes play a significant impact. This study focuses on the procedures used, the value of, and risks associated with, gene-related medical interventions.*

1. INTRODUCTION

The primary physical and functional unit of heredity is the gene. A gene is an ordered series of nucleotides that are situated in a given location on a certain chromosome and encode a particular functional product (i.e., a protein or ribonucleic acid molecule). A "biological unit of heredity" is referred to as a gene. Unique characteristics, such as eye color and hair color and texture, are inherited from the parents (Chew *et al*., 2018). The amount of oxygen the blood can carry, the child's IQ, and other factors are also determined by them, such as the child's gender.

The lengthy strands of a substance termed deoxyribonucleic acid that make up genes are found in one file within the chromosomes. Nucleotide subunits of deoxyribonucleic acid, or DNA, are responsible for encoding the genetic message. The chromosomes of a human cell contain about three billion pairs of nucleotides (Cheng *et al*., 2021). Because every individual has a different sequence of nucleotides in their genetic composition, this is what distinguishes us from one another.

Every human cell is thought to have roughly 30,000 genes, according to scientists. A sickness, a physical impairment, or a shorter lifespan may be caused by a mutation or flaw in any one of these genes. Just like blond hair from the mother or brown eyes from the father, these mutations can be passed down through generations. But with gene therapy, treating or curing physical ailments or genetic diseases brought on by these mutations might become a reality (Goswami *et al*., 2019).

A research method used to replace, alter, or add functional genes to those that are damaged or faulty. Specific base sequences called genes are responsible for encoding the instructions needed to produce proteins. Despite the fact that genes receive a lot of attention, proteins actually carry out the majority of life's tasks and even make up the majority of cellular structures. Genetic abnormalities can result from genes that have been changed so that the encoded proteins can no longer perform their typical roles (Goswami *et al*., 2019).

Gene therapy has brought permanent cures for ailments that were previously simply temporary treatments. For a very long period, gene therapy did not work; however, in recent years, effective and long-lasting treated cases have been recorded. For a variety of hereditary ailments, including blood abnormalities, immunological deficiencies, vision issues, regeneration of nerve cells, metabolic disorders, and different types of cancer, promising results have been attained (Zhang *et al*., 2018).

Approximately 2000 clinical trials have been completed or are ongoing on various individuals as of this writing, and many more are in the planning stages. However, the invention of gene therapy vectors and the clinical development of these vectors are moving forward quickly (Thakur *et al*., 2018). After describing the method of gene therapy, this study will cover some of the notable recent successes of clinical gene therapies by outlining various strategies and introducing general tools, such as vectors—the most crucial gene therapy tool.

Diseases that cannot be treated with conventional medicine may be treated with gene therapy. It is done either by altering a damaged gene or by introducing one or more nucleic acids into the cells of the patient. Development of gene therapy vectors, optimization of gene delivery in both in vivo and in vitro settings, and improvement of clinical experience are the key drivers of investment in gene

therapy for human diseases (Spicer *et al*., 2018). As a cutting-edge technology, gene therapy has expanded to treat a variety of conditions beyond just genetic abnormalities. In fact, the promise of modifying chimeric antigen receptors (CAR) on T cells for the treatment of leukemia led Science magazine to name cancer immunotherapy as the most significant scientific advance of 2013(Goswami *et al*., 2019).

Gene administration to non-dividing cells and tissues (post-mitotic cells) in vivo or gene delivery to autologous cells outside the body (ex vivo), in which the gene is given to the patient through adoptive transfer, are both effective methods for clinical gene therapy. Adeno-associated viruses (AAVs) have demonstrated the highest clinical success in in vivo gene transfer among viral vectors. They can target a variety of cells and tissues because to the large diversity of serotypes and capsids (Spicer *et al*., 2018).

Clinical gene therapy in vitro concentrates on the transfer of genes to autologous hematopoietic stem cells (HSCs) for the treatment of various illnesses, particularly hematologic ones, and to other blood cells such various types of T lymphocytes for immunotherapy. The efficiency of retroviral vectors on hematopoietic cells has been established. Retroviral vectors, including -retroviral and lentiviral variants, can insert their greatest amount of genetic material into the genome of the target cells. Lentiviral vector use and methodologies changed as a result of early adverse impacts of -retroviral vectors, and this led to the development of a better, more dependable, and more efficient preclinical method for delivering genes to non-diverging cells (Dunbar *et al*., 2018).

The European Medicines Agency has given the green light to Glybera as the first gene therapy product. It was the first significant step toward creating a gene-based medication (Dunbar *et al*., 2018). Clinical gene therapy has recently made considerable strides in treating a variety of single-gene disorders, such as early immunological deficiency, hemoglobinopathy, hemophilia B, neurological diseases, ophthalmic diseases, and biological cancer treatment (apart from enclitic cancer treatment) (Prondzynski *et al*., 2018).

DNA is used as a medication in gene therapy to cure illness. Additionally, a gene or genes within cells are altered as part of an experimental medical procedure to produce proteins that alter the way those cells function (Hidai and Kitano, 2018). Its term comes from the notion that DNA can be utilized to enhance or modify genes within specific cells. In order to replace a defective gene, the most popular type of gene therapy uses DNA strands that encode functional therapeutic genes. In gene therapy, the DNA for a therapeutic protein is packaged within a "vector" that is used to deliver the DNA to the body's cells. The cell machinery now uses the DNA within to express itself, which causes the development of a healing protein that heals the patient's illnesses (Prondzynski *et al*., 2018).

In an effort to treat and cure some well-known genetic illnesses, the majority of which are treated ineffectively, the notion of gene therapy was initially developed. Gene therapy's original intent was to replace or repair a damaged gene with a healthy one in order to reduce or eliminate disease or defect symptoms (Hidai and Kitano, 2018). Though approximately 75% of all clinical trials are focused on cancer, AIDS, Alzheimer's, diabetes mellitus, and arthritis treatments, which all entail genetic vulnerability to sickness, researchers have expanded their study to cure various types of diseases in addition to inherited genetic problems (Calos, 2018).

Despite the seemingly endless potential of gene therapy, numerous technological issues have prevented study. To consider gene therapy an unambiguous success and to justify treating a significant number of individuals, the majority of clinical trials have not led to enough improvement in the patients' underlying disease (Esrick and Bauer, 2018). Critics who fear that the science might advance too far have also been alarmed by gene therapy's amazing potential. They point out that gene therapy may present affluent families with genetic enhancing options that are not available to the poor. For other skeptics, gene therapy's potential to reduce the human gene pool, with unknown and potentially detrimental repercussions, is even more concerning (Morrow, 2018).

2. GENE THERAPY

2.1 Definition of Gene therapy

Gene therapy is a "new strategy to treat, cure, or eventually prevent disease by modifying a person's gene expression," according to the American Medical Association (Guan *et al*., 2019). DNA, which makes up genes, contains the instructions needed to produce the proteins the body needs to function properly. Genetic illnesses can result from gene mutations that cause these proteins to be produced incorrectly or not at all. In order to (re)establish normal function, defective genes that cause disease are repaired, dis-activated ("turned off"), or replaced in gene therapy (Guan *et al*., 2019).

The gene is typically delivered via a carrier, or "vector," which frequently takes the form of a modified virus (one that does not cause disease). The virus then incorporates its genetic material into the human cells after being introduced to the human tissue, either by injection, intravenously, or outside the body in a lab. Gene therapies are often invasive because of this (the majority via intravenous, subcutaneous, intraperitoneal or intramuscular injection). The new gene will produce a functional protein, assuming the treatment is successful (Nance *et al*., 2020).

Around 4,000 diseases, including cancer, AIDS, cystic fibrosis, Parkinson's and Alzheimer's diseases, amyotrophic lateral sclerosis (Lou Gehrig's disease), cardiovascular disease, and arthritis have been related to gene problems, according to the American Medical Association (Nance *et al*., 2020). Therefore, the potential of a successful gene therapy treatment could have a significant impact on millions of lives. However, there are numerous obstacles to be overcome. The science is difficult, especially as we move away from illnesses caused by a single gene.

2.1.1. Cellular, Tissue and Gene Therapies

Gene treatments, cell therapies, and tissue engineering techniques are frequently categorized together with one another under the headings of "regenerative medicines" or "advanced therapeutics." When it comes to becoming commercialized, several other methods have advanced more than gene therapies: There are now 13 medicines approved by the FDA's Office of Tissue and Advanced Treatments (OTAT), formerly known as the Office of Cellular, Tissue and Gene Therapies, none of which are gene therapies (Manunta *et al*., 2018). Seven regenerative medicine products have been authorized for marketing in the European Union. The only one of them, ChondroCelect, a tissue-engineered therapy, has, however, only been successful in obtaining national reimbursement in three nations (Spain, Belgium, and the Netherlands) (Manunta *et al*., 2018). Products used in gene therapy are considered therapeutic biological products by the FDA. They are governed by the Public Health Service (PHS) Act's licensing rules and the Federal Food, Drug, and Cosmetic Act's drug regulations (FFDCA). A biologic licensing application (BLA) is necessary for approval (Nance *et al*., 2020).

2.2. The Gene Therapy Pipeline

Gene therapy is a promising topic for medication research since it can treat a serious disease from the inside out with the appropriate target and strategy. Gene therapy can "correct" the underlying problem and/or offer a route to generating the functional protein for some illnesses where known genetic abnormalities cause insufficient or non-functional protein production (Nance *et al*., 2020). The cystic fibrosis conductance regulator (CFTR) gene, for instance, is prone to genetic abnormalities that alter mucous discharges and cause respiratory dysfunction, recurrent respiratory tract infections, high morbidity, and early mortality. Because of the potentially fatal nature of the ailment and the understood biology behind the illness, cystic fibrosis has long been a focus for the development of gene therapies (Deev *et al*., 2018).

A cure could result in increased life expectancy, higher quality of life, and lower healthcare costs (medications, physical therapy, lung transplantation, etc.). The number of potential molecular targets for gene therapy is increasing as our knowledge of the human genome deepens, and with it, our hope for correcting the genetic pathways underlying illnesses for which there have been only modest or no breakthroughs (Deev et al., 2018). At the ICER Policy Summit, there was broad consensus that all parties involved must work together to make these potentially game-changing medications available to the people who need them most.

Currently, gene therapy is still viewed as "experimental" (Goswami *et al*., 2019). The first gene therapy, Glybera from UniQure to treat lipoprotein lipase deficiency, received FDA approval in 2012 but was not authorized for use in the EU. Due to its $1.4 million price tag, it has only been paid for usage in one patient in Europe to date (Ylä-Herttuala, 2019). As of 2016, another treatment, Strimvelis for children with severe combined immune insufficiency, is also authorized in Europe, but not yet in the US (Goswami *et al*., 2019).

A new analysis from Pharma projects (Boliter, 2018) reveals that the number of gene therapies in development has expanded significantly over the last 2-3 years, despite the FDA still having not approved a gene therapy. There are currently 23 active phase III gene treatments being developed out of these. Up to 12-14 gene therapies that are currently in active phase III development (about 5-6 non-cancer therapies and 7-8 cancer therapies) may submit a new license application in the following few years if one assumes the same success rates as for conventional therapies at this stage (Manunta *et al*., 2018).

The current front-runner for the treatment of hereditary retinal illness brought on by an RPE65 mutation is voretigene neparvovec from Spark Therapeutics. The potential for this therapy to produce clinically significant and long-lasting improvements in retinal sensitivity is shown by the findings of a pivotal phase 3 study. This therapy would enable patients to have better functional vision, which would, for instance, improve mobility. The FDA Biologics Licensing Application process has already started for Spark Therapeutics, and the company hopes to finish it sometime in early 2017 (Manunta et al., 2018).

2.3 Approaches to Gene Therapy

2.3.1 Gene modification

The following techniques have been applied by researchers to fix flawed genes:

i. Gene replacement therapy: Using homologous recombination to replace a native gene with a synthetic gene.
ii. Modifier gene therapy: Restoring a faulty gene's normal function through targeted reverse mutation
iii. Modification of a certain gene's expression

2.3.2 Gene transfer method

To transfer genes, there are three different techniques: physical, chemical, and biological.

A specific cell line receives a gene transfer

Somatic gene therapy and sex cell gene therapy are the two main divisions of this field.

The use of genetic engineering in the most appropriate way (gene injection)

Other types of genetic engineering involve targeting certain genes and eradicating them using nucleases created by TAL effectors, I-CreI homing endonucleases, or zinc finger nucleases. In human clinical trials, this approach is now being applied (Urnov *et al*., 2018)

2.4 Vectors in Gene Therapy

2.4.1 Retroviruses

The class of viruses known as retroviruses, which includes the human immunodeficiency virus (HIV), are capable of converting RNA genomes into double-stranded DNA and integrating into host cell chromosomes. The integrase enzyme, which is used in retrovirus-based gene therapy, has the ability to insert the virus genome wherever in the host genome (Tejada *et al*., 2018). The function of the affected gene will be compromised (insertional mutation) if the genetic material is inserted into the midst of one of the host cell's major genes (Tejada *et al*., 2018).

A cancerous cell division will result from gene insertion during the cell division process. Zinc finger nucleases and specialized sequences, such the beta globin regulatory region, which direct the insertion of the gene into specific places of the chromosome, have helped to partially alleviate this problem (Tejada *et al*., 2018).

2.4.2 Adenoviruses

Human infections of the respiratory, gastrointestinal, and ophthalmic systems are brought on by this group of viruses, which have double-stranded DNA genomes. The DNA molecules of these viruses are inserted into the host cell's DNA during infection, but the genetic makeup of the adenovirus does not mix with that of the host cell. Instead, the DNA molecule stays inside the nucleus of the host cell, where it is transcribed like any other gene. Adenoviruses differ solely in that their foreign genes do not duplicate when the host cell divides; as a result, the cells produced by cell division do not include any new genes. Adenoviruses must therefore be injected again in order to treat a developing cell population (Lowenstein and Castro, 2018).

2.4.3 Adeno-Associated Viruses

AAVs are a tiny class of viruses having uncoated, single-stranded DNA. They have the capacity to infect cells with constitutive expression in both proliferating and non-dividing tissues (Meyer *et al*., 2017). These viruses are a suitable choice for gene therapy since they can exist in cells in both lysogenic and lytic forms. These viruses' lack of pathogenicity for humans in the absence of auxiliary viruses like adenoviruses and herpes viruses is another salient characteristic. These viruses have the ability to insert their genetic material into a specific spot on chromosomes without the aid of additional viruses (Meyer *et al*., 2017).

2.4.4 Herpes Simplex Viruses

This group of viruses infects a specific subset of brain cells and has double-stranded DNA. Cold sores and fever blisters are symptoms of type 1 herpesvirus infection, a common disease in people (Kassiotis and Stoye, 2017). Human neurotropic herpes simplex virus is mostly used in the neurological system for gene transfer. The wild HSV-1 virus has the ability to infect neurons while evading the host's immune response; nonetheless, this virus may become inactivated and cause a lytic cycle of viral reproduction. As a result, the most common HSV-1 strain is a mutation that cannot replicate (Meyer *et al*., 2017).

2.4.5 Cis and trans-acting elements

There is always a "transfer construct" in replication-defective vectors. The "transgene" or gene that will be transferred is carried by the transfer construct. The packaging sequence, replication repeats, and, if necessary, reverse transcription priming sequences are all carried by the transfer construct together with the other sequences required for the viral genome's basic function (Hayward, 2017). Since the target gene and the viral genome must be on the same piece of DNA, these are known as cis-acting elements (Hayward, 2017).

2.5 Non-viral Methods

Nowadays, non-viral approaches are better than ones that are viral. Only 2 of their benefits include ease of manufacture at a large scale and fewer immune reactions from the host (host immune system responses). In the past, this method's poor gene expression and high transfection level were thought to be drawbacks. However, recent advancements in vector technology have enabled the development of compounds and methods that are as effective as viral ones (Artusi *et al*., 2018).

2.5.1 Ormasil

One other non-viral technique is the usage of Ormasil, which is made of silica or modified organic silicate. Silica has proven to be a viable choice for gene transfer due to its comparatively simple processing. Due to its low toxicity, silica is used in gene therapy most frequently when combined with amino silicones in the form of nanoparticles (Yamamoto *et al*., 2017). However, administration with serum lowers the effectiveness of this approach because serum protein reactions act as a limiting factor (Ramamoorth and Narvekar, 2019).

2.5.2 Injection of Naked DNA

The simplest non-viral transmission technique is injection of naked DNA. Gene expression is substantially lower with this approach than with other ways, despite the fact that clinical trials of it have been effective. In addition to plasmid tests, tests with naked PCR products have also been conducted (Yamamoto *et al*., 2017). In general, bare DNA is poorly absorbed by cells. The development of

new techniques, including as electroporation, sonoporation, and the use of the "gene gun," in which DNA coated with gold particles is injected into a cell with helium gas at high pressure, has resulted from research efforts to increase the efficiency of DNA uptake.

2.6 Physical Methods for Improving DNA Transfer

2.6.1 Electroporation

High-voltage brief pulses are used in the electroporation technique to transfer DNA from cell membranes. The membrane develops temporary small pores as a result of an electrical shock, which allows nucleic acid to pass through. Numerous cell types can be electroporated, however due to high rates of cell death, its usage in clinical settings has been constrained (Ledgerwood *et al*., 2017)

2.6.2 Gene Gun

Another physical technique for transferring DNA is the use of particle bombardment, or gene guns. This technique involves coating the DNA with gold particles and putting it within a machine that can provide the necessary force to enter the cell. However, if the DNA is positioned incorrectly in a genome, for example, in a tumor suppressor gene, it may cause a tumor to develop (Ledgerwood *et al*., 2017). When HSCs were infected with a retrovirus containing the modifying gene in clinical trials on patients with X-linked severe immunodeficiency (X-SCID), 3 out of 20 patients were successfully treated for T cell leukemia.

2.6.3 Sonoporation

Ultrasonic frequency is used in sonoporation to insert DNA into a cell. DNA mobility results from this event, which is regarded as ultrasound cavitation in the cell membrane (Ledgerwood *et al*., 2017).

2.6.4 Magnetofection

In order to expose the DNA-containing substance to just one cell layer during magnetofection, magnetic particles are complexed with DNA and placed under the cellular tissue culture container with a magnet (Ledgerwood *et al*., 2017). This approach, which is predicated on the idea of targeted medicine delivery, functions by attaching the therapeutic gene to the magnetic nanoparticles. Complex deposition and transfection rate are both accelerated by the electromagnetic field gradient produced by the earth beneath the cell culture medium (Ji *et al*., 2017).

The gene-magnetic complex is given intravenously in vivo, where it is absorbed and moves toward the target with the aid of powerful external magnets. The gene is ultimately extracted from the magnetic particles using intermolecular restriction enzymes, charge interaction, or matrix breakdown (Hayward, 2017). This technique is frequently used in laboratory investigations to transfer a gene to primary cells and other cells where other approaches are challenging to do so (Yamamoto *et al*., 2017).

2.7 Chemical Methods for Improving DNA Transfer

2.7.1 Oligonucleotides

Gene therapy employs synthetic oligonucleotides to silence and inactivate disease-related genes. The transcription of faulty genes is hampered by the use of specialized antisense for the target gene. The use of siRNA, which causes the breakdown of a particular sequence of the faulty gene's mRNA to prevent translation and, consequently, expression, is another technique (Tazawa *et al*., 2017).

2.7.2 Lipoplex and Polyplex

Polyplex is a term used to describe a mixture of DNA and polymers. The majority of polyplexes contain cationic polymers that are created through particle aggregation as a result of interactions between polyplexes. DNA should be shielded from harm and a positive charge to improve fresh DNA transport to the cell. As a result, lipoplexes are created using neutral and anionic liposomes as synthetic vectors (Tazawa *et al*., 2017).

2.7.3 Dendrimers

A globular, branching macromolecule, the dendrimer. Many characteristics of the final particle structure are governed by the particle surface, which can be charged in a variety of ways. A temporary nucleic acid linkage forms with the cationic dendrimer in the presence of genetic material like DNA or RNA as a result of the additional charge. Through endocytosis, the nucleic acid-dendrimer complex enters the cell (Dai et al., 2017).

2.7.4 Hybrid Methods

Each gene transfer technique has its own drawbacks, thus hybrid methods—which are actually fusions of various techniques—are being developed to address these issues. A typical hybrid approach is a virosome, which combines a liposome with an inactive HIV or influenza virus (Wood et al., 2006) More effectively than either the viral or the liposome techniques alone, this hybrid approach to gene transfer in respiratory epithelial cells. The fundamental idea behind this technique is to combine several viral vectors with cationic liposomes or hybrid viruses (Dai *et al*., 2017).

3. PROBLEMS ASSOCIATED WITH GENE THERAPY

The following highlights and discusses a few of the issues with gene therapy. The issue includes immune response stimulation, multigene disorders caused by the presence of multiple genes, the short half-life of gene therapy, immune response, viral vector issues, multigene disorders, the potential to cause tumors (insertional mutagenesis), manufacturing and distribution, moral quandaries, and clinical evidence of effectiveness and safety.

3.1. Stimulation of immune response

Due to the virus's presence inside the body and the potential for viral vectors to be pathogenic (in one scenario, the viral vector may enhance the virus's capacity to cause illness), the gene that a virus has injected may trigger immunological reactions (Dai *et al*., 2018).

3.2. Generation of genetic disorders due to the presence of multi-gene

Even if the genetic material does penetrate the target cell, it might not be deposited in the genome's proper location (Dai *et al*., 2018).

3.3 Short-lived nature of gene therapy

The therapeutic DNA must remain functioning in the targeted cells for gene therapy to result in a lasting cure for any ailment, and the therapeutic DNA-containing cells must be stable and long-lived (Dai et al., 2018). Gene therapy cannot produce any long-term advantages due to the difficulty incorporating therapeutic DNA into the genome and the propensity of many cells to divide quickly. Multiple rounds of gene therapy will be required for patients (Dai *et al*., 2018).

3.4 Immune response

The immune system of humans has developed to attack invaders whenever they are introduced into the body. There is always a chance that immune system stimulation could lessen the efficiency of gene therapy. Furthermore, it is far more challenging to repeat gene therapy in patients due to the immune system's heightened response to intruders that it has already encountered (Sharma *et al*., 2017).

3.5 Problems with viral vectors

Viruses, the preferred carrier in the majority of gene therapy research, present the patient with a range of potential concerns, including toxicity, immunological and inflammatory reactions, as well as problems with gene regulation and targeting (Sharma *et al*., 2017). Additionally, there is always the worry that the viral vector may regain its capacity to spread disease once inside the patient.

3.6 Multigene disorders

Gene therapy works best for diseases or disorders that result from mutations in a single gene. Unfortunately, genetic variants have a large role in the development of some of the most prevalent ailments, including heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes (Sharma *et al*., 2017). Disorders with several genes or multiple factors, such as those listed above, would be very challenging to adequately treat utilizing gene therapy (Sharma *et al*., 2017).

3.7 Chance of inducing a tumor (insertional mutagenesis)

A tumor may develop if the DNA is incorporated in the incorrect region of the genome, such as a tumor suppressor gene. When hematopoietic stem cells were retrovirally transduced with a corrected transgene in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, 3 of 20 patients developed T cell leukemia as a result (Lawler *et al*., 2017). Adding a functioning tumor suppressor gene to the DNA to be integrated is one potential remedy, but this has drawbacks as well because lengthy DNA is more difficult to integrate effectively into cell genomes (Lawler *et al*., 2017).

3.8 Manufacturing and distribution

Gene treatments differ from small and large molecule medications in that they are individualized and have a shorter shelf life and less stability (Bailey *et al*., 2019). As a result, goods cannot always be produced in large quantities. Therefore, there can still be delivery-related practical issues to deal with even if the manufacturer is successful in obtaining market permission and payment. Concerns have also been expressed about the possibility that unclear manufacturing and quality standards could hinder progress by causing ineffective product development (Lawler *et al*., 2017).

There are, in reality, few, if any, industry standards for the production of goods used in cell and gene therapy, according to Bailey *et al*. (2019). In uncommon diseases with no examples, this is likely to be very challenging (van Schothorst *et al*., 2018). While establishing excellent manufacturing practice and lowering costs through experience and standardization, there may be a trade-off between allowing flexibility in the manufacturing process to take into account the patient-specific nature of the products. The

production of gene "biosimilars" could become more challenging in the future due to the distinctive character of gene therapy manufacturing.

3.9 Ethical dilemmas

Gene therapy ethical problems include I the difficulty of denying coverage due to cost for a therapy that appears to be effective for patients with significant morbidity; and (ii) the challenge of conducting a randomised clinical trial with a subpar current standard of care, when the new therapy appears to be performing well. These ethical problems are similar to those for cancer treatments for small populations (Ugwu et al., 2019). Withholding experimental treatment from trial participants in situations where there are no effective alternatives and the intervention is for a life-threatening ailment may be considered unethical due to a lack of clinical consensus (Ugwu *et al*., 2019).

3.10 Evidence of Clinical efficacy

Gene therapy ethical problems include I the difficulty of denying coverage due to cost for a therapy that appears to be effective for patients with significant morbidity; and (ii) the challenge of conducting a randomised clinical trial with a subpar current standard of care, when the new therapy appears to be performing well. These ethical problems are similar to those for cancer treatments for small populations (Ugwu *et al*., 2019). Withholding experimental treatment from trial participants in situations where there are no effective alternatives and the intervention is for a life-threatening ailment may be considered unethical due to a lack of clinical consensus (Ugwu *et al*., 2019).

Invasive methods of administration may necessitate sham procedures in order to perform blinded RCTs when a placebo or comparative arm is possible. These "sham" operations can be unethical and could prevent completely blinded placebo-controlled research designs (Xu *et al*., 2019).

It can be difficult to find the appropriate comparator to compare gene therapy to, particularly when there are no other therapies available or when the therapy has a major impact on clinical practice. According to a report from the European Commission, there may not be a defined clinical pathway (i.e., no conventional "usual care") or established assessment of clinical outcomes in situations when there is no standard treatment available (Xu *et al*., 2019).

Trials are rarely longer than a few years, thus they cannot give long-term data. Many have a longer follow-up period during which patients are observed, but as one interviewee noted, manufacturers would need to keep track of patients for a long time in order to provide data showing that these treatments are permanent, or at least that the long-term clinical benefit outweighs the short-term risks (Knopp *et al*., 2018).

It may be challenging to power studies effectively to assess the desired health outcomes due to the limited patient numbers and perceived ethical and statistical benefits of crossover trials (Knopp et al., 2018). In order to evaluate the important clinical and health endpoints, crucial trials may therefore be forced to rely on surrogate outcomes that require extrapolation. In estimations of effect, this can be a significant source of uncertainty. Hettle *et al*. (2016) conducted a review of the literature (of works and statistical studies on the evidence for the use of surrogate endpoints in medicine) and discovered that trials using surrogate outcomes typically report larger estimates of treatment effect (28%–48%) than trials using clinical endpoints. Furthermore, adequate surrogate outcomes for gene therapies might not already exist, necessitating the invention and validation of outcome metrics that are not yet well-known to patients, doctors, or payers.

The lack of a head-to-head comparison when comparable data are collected from sources other than an RCT can increase the uncertainty surrounding estimates of relative effectiveness. According to Hettle *et al*,. (2016) 's analysis of the literature, single arm trials are likely to yield overly optimistic estimates of benefit unless historical control data are available. As previously stated, there will be a great deal of ambiguity if adaptive licensing with surrogate markers is adopted, necessitating confirmation investigations after regulatory approval (Kim *et al*., 2017).

The dangers and advantages of a product may occasionally differ based on the delivery protocol and, if appropriate, the surgical team's level of expertise, further complicating the evaluation of clinical effectiveness (Abou-El-Enein *et al*., 2016a). High individual and center-level variance in response could result from this, which would have an impact on how generalizable efficacy and safety estimations are. There may be a "learning effect" over time that helps to increase the efficacy of the treatment if the surgical team's ability is an important consideration. These problems can occur in any surgical RCT, yet they can nonetheless raise additional doubts about clinical efficacy.

3.11 Safety evidence

Gene treatments have their own unique set of safety challenges; when administered by viral processes, they have the potential to be tumorigenic and to promote proliferation in tissues that were not intended to be affected. Additionally, they may trigger immunological responses that call for immunotherapy, raising the dangers overall (Abou-El-Enein *et al*., 2016a).

Gene therapies are very new to medicine, so there is no extensive long-term data with which to assess the likelihood of major safety implications that might occur years after the first therapy phase. Several interviewees emphasized the issue of unknowable long-term safety consequences: it was noted that the FDA has been pushing for lengthy trials to gather as much information as possible, but the truth is that we might not know the true effects of some of these therapies for 5–10 years after administration (Sengillo *et al*., 2017).

Given that we don't fully understand the effects of changing the gene, there is uncertainty over the possibility of injury (Sengillo *et al*., 2017). At the ICER Policy Summit meeting, additional worries were expressed about how using these medicines now would have unanticipated effects on future generations. Manufacturers made it clear that this is not anticipated to be the case because many of the gene therapies in development boost or supplement already-existing genes rather than altering germ line DNA sequences (Sengillo *et al*., 2017).

4. PROSPECTS OF GENE THERAPY

Gene therapy has a wide range of potential applications. We are all aware that gene therapy is not a panacea. Although it is not always effective, it shows that a sickness can be eliminated for a person and their descendants in the future. As a result, it is treated not only in the current generation but also in succeeding generations (Ruella and Kenderian, 2017).

Gene therapy also has the ability to "mute" a gene, as was the case with a subject who had HIV but had not yet progressed to AIDS; by utilizing gene therapy to "quiet" the disease before it manifested, researchers were able to spare the individual the misery and suffering of the condition. I think the skeptics and individuals who are wary of this approach would alter their minds if they ever had to deal with cancer or a child born with a hereditary illness. Even if gene therapy was their or a loved one's last option, as it is for many gene therapy patients, these skeptics would almost surely select it (Khan *et al*., 2018). A group of medical professionals in Paris reported the findings of a study involving two kids with SCID-XI, a severe form of combined immunodeficiency that had forced them to live in isolation, in 2000.

After inserting a therapeutic gene (the c cytokine receptor component) into the patients' lymphocytes ex vivo using a MoMLV vector, these researchers amplified the cells before giving them back to the patients. Each patient was able to leave the hospital and return to their regular life (Stepanenko and Chekhonin, 2018). Gene therapy holds the promise of curing or preventing inherited conditions like cystic fibrosis and holding promise as a treatment for cancer, AIDS, heart disease, and other conditions. This potential will go a long way toward ensuring better health for future generations, enhancing immune function in those with immune deficiency disorders, increasing farm animal productivity, and producing genetically modified animals (GMOs), which will further aid in medical and biomedical research (Carding *et al*., 2017).

4.1 Applications of Gene Therapy

4.1.1 Parkinson’s Disease

Independent reports have established the efficacy of gene therapy in treating Parkinson's disease (PD). For instance, one of the suggested techniques raises the brain's concentration of GABA, a neurotransmitter whose deficiency causes Parkinson's disease (PD). In a study involving 45 volunteers with severe Parkinson's disease, tubes were inserted in the parts of the brain connected to movement (Carding *et al*., 2017). Injections of viruses with the gene that boosts GABA production were given to half the subjects, while the other half received a harmless saline solution (as the control group). After six months, those who received gene therapy displayed a 23% improvement in their capacity to move, which was twice the improvement seen in the control group (Carding *et al*., 2017).

The study under discussion was a randomized controlled experiment to look at how gene therapy might help with advanced PD symptoms. In the study, cells in the base ganglia, a collection of cerebral regions controlling movement, were given genes that produce the chemical agent glutamic acid carboxylase (GAD). A chemical messenger termed GABA was produced in greater quantities as a result of the GAD gene translocation. Parkinson's patients have lower levels of GABA in some basal ganglia regions (Suwanmanee et al., 2017)

The cell-to-cell transfer of the protein mass has been acknowledged as a mechanism of harm that extends across the central nervous system in addition to the disease-causing pathways that have so far been identified. Growing understanding of the immune system's role in PD's primary cause, the disruption of transcription and translation at the disease's inception is another development (Taymans *et al*., 2018). It will be feasible to comprehend the fundamental causes of the disease as our knowledge of PD grows. The blood-brain barrier, which prevents the admission of medications with specific target cells in the brain, must be broken (van der Brug et al., 2017). Recent research on LRRK2 inhibitor in mammals other than humans suggests that long-term usage of the drug or genetic modification of the target tissue could have substantial side effects. (Fuji *et al*., 2016; Suwanmanee *et al*., 2017).

4.1.2 Alzheimer's Disease

There are currently no effective treatments for the majority of nervous system problems, including mental disorders, and Alzheimer's disease (AD) is the leading global cause of dementia. All frontotemporal dementias (FTDs) and AD are collectively referred to as tauopathies because they are brought on by the brain's excessive buildup of damaged tau. Recombinant AAVs (rAAVs), a recent advancement in gene therapy-based methods, have given researchers new tools for studying AD and other neurological illnesses (Combs *et al*., 2016)

Patients with Alzheimer's disease were subjected to a clinical trial of nerve growth factor (NGF) gene therapy in 2001 to see if dying neuronal cells in AD, after the onset of the disease, still had the capacity to react to the NGF. A dysfunctional nerve gene was initially introduced into an adult during the first attempt. Based on the findings, NGF was able to stimulate the brain's deteriorating neuronal cells in AD. Every patient responded to NGF nutritionally; axonal sprouts were grown in the direction of the NGF source. To determine the extent of treatability, the brains of three patients who underwent one-way gene transfer were studied. It was discovered that cholinergic neuronal hypertrophy occurred on the side treated with NGF ($P>0.05$).

Functional indicators and cellular signaling were stimulated in the cases of the two patients who received AAV2-mediated and NGF gene transfer treatments. Both neurons with and without Tau damage—a protein that promotes microtubule stability—expressed NGF, indicating that dying cells may be treated with gene therapy to activate the cellular signaling system. No negative effects connected to NGF were noticed. Over ten years, which was longer than anticipated, NGF-induced sprouting persisted (Tuszynski *et al*., 2019).

The Daily Mirror said that researchers used the novel strategy of direct medication delivery to the brain to mute a gene related to AD. Exosomes, which are tiny particles that are released into cells, were employed by researchers to carry drugs to the rat brain. Exosomes offer a wide range of possible uses for carrying particular genes in the brain and can therefore be utilized in gene therapy, according to the findings of studies on rats. BACE1 is one of these genes. The scientific community gave this work a lot of attention, but it was only a preliminary study, and this technology has not yet been tested on human cells for a variety of reasons, including ethical concerns.

The deterioration and loss of cells are common features of many neurological disorders. In both the central and peripheral nervous systems, neurotrophic factors—proteins that promote cell proliferation during development and act as neuroprotectors in a variety of neurological diseases—are excellent candidates for gene therapy (Kalimuthu *et al*., 2017). It has received a lot of attention because GDNF, a neurotrophic factor in the brain, protects the midbrain's subgroup of dopaminergic (dopamine-producing) neurons from damage. In PD patients, these neurons deteriorate. GDNF generation could be a therapeutic objective (Kalimuthu *et al*., 2017).

According to numerous research,GDNF induction into the brain has proven effective in curing animals of the behavioral symptoms of Parkinson's disease (PD) and halting the degeneration of nigrostriatal dopaminergic pathways. The study's authors hypothesized that gene therapy is more efficient since it only temporarily releases GDNF from the cerebral cortex and stops further brain structural deterioration. A new lentiviral vector designed specifically to infect the brain and spinal cord has been created based on the equine infectious anemia virus (EIAV). The majority of the infected cells have a neurological morphology, and GDNF administration via VSVG-EIAV has been effective in PD-affected mice.

The use of HSV vectors, which can be used in posterior and peripheral transfers to the posterior root of ganglion cells, is the main topic of research on treating painful areas. According to a study on the use of HSV as a GDNF vector, pain was successfully relieved and neurochemical changes were minimal. Although there are several benefits to this delivery strategy with little invasion, the virus can still infect brain cells even through a skin scrape. The following opportunities exist for employing the HSV system:

i. Viruses are linked to toxicity and immunological reactions, however employing these new techniques, viruses can be improved and their poisonous and immune-reactive components can be removed.

ii. During the incubation phase, there is a very small expression of the virus' replication in the infected cells.

Numerous techniques and models related to pain have used various spinal cord inducer virus mediator molecules with success. An AAV-BDNF neuronal agent's protective effects were described by Eaton et al. The peripheral neuropathy model has not yet been adjusted to include GDNF as an intra-spinal cord inducer. AAV and Lv viral expression vectors have been used in a number of experiments employing intra-spinal injections of GDNF in ALS models and ventral root avulsion (Patil *et al*., 2012).

4.1.3 Cystic Fibrosis

The condition known as cystic fibrosis (CF) slowly kills the lungs. Infection of the respiratory system, inflammation, deformity, and blockage are some of its symptoms. There are potential benefits to the direct introduction of the CFTR gene into respiratory tract epithelium cells as the target tissue. The host's lung's physical and immune barriers, meanwhile, pose certain obstacles to the gene's effective translocation to the respiratory system. Research on CF has been sparked by advancements in tissue engineering, gene transfer techniques, and animal models (Oakland *et al*., 2017)

4.1.4 Diabetic Neuropathy

Researchers found that gene therapy holds promise for treating diabetic polyneuropathy, a common condition brought on by persistent diabetes. Patients with diabetic neuropathy may benefit from intramuscular injection of a vascular endothelial growth factor (VEGF) gene, according to researchers in Boston. 39 patients received three VEGF injections in one leg during this trial, along

with 11 placebo recipients. Diabetic neuropathy can cause leg and plantar discomfort, weakness, and balance issues. A foot ulcer may not be discovered due to a loss in touch perception, which may necessitate an amputation. Most patients experienced neuropathy that was fairly severe and had slim chances of healing. The VEGF gene that was employed in this trial was active and present without any packaging in the virus, which is a tremendous and significant benefit, according to Dr. Allan Ropper, Executive Director of the Department of Neurology at Brigham and Boston Hospitals. The study suggested that this type of gene transfer would be somewhat safe, but more investigation with a bigger study population is required before it can be used as a significant treatment (Patil *et al*., 2019).

4.1.5 Gene Therapy for Cancer Treatment

Over the past two decades, advances in human genomics have demonstrated that cancer is brought on by aberrations in the somatic cells' host genome. These successes have encouraged numerous cancer researchers to explore genetically modified and altered therapies to treat cancer and possibly find a cure for the illness. Examples include gene therapy utilizing non-viral or viral (or bacterial) vectors, immunomodulation (stimulation of the immune system against tumor cells), tumor cell manipulation to shrink tumor tissue, and enhancing antigens for improved tumor detection by the host immune system (Machiels *et al*., 2019).

In general, there aren't many treatments that have few negative effects. By integrating a retrovirus with the host genome, which carries the risk of mutagenicity or malignancy, immunological response against viruses, tumor formation, drug resistance, or illness relapse, new generation viral or non-viral vectors considerably reduce these risks.

Many vaccinations, genetically altered immune cells, and tumor-specific antibodies have been created and are already on the market; many more are undergoing clinical trials. Along with other cancer treatments including surgery, radiation, and chemotherapy, gene therapy is anticipated to play a significant position in a comprehensive approach to treating cancer. In order to provide a complex treatment that is specific to each patient's needs, the kind and state of gene therapy are decided based on the individual genome components, tumor features, genetics, and host immunological status (Amer, 2016).

In gene therapy for cancer, viral vectors are the primary vehicle for gene transfer. A more promising future is offered, in particular, by oncolytic viruses that specifically attack and kill cancer cells. The use of stem cells for tissue regeneration, the ability to edit and modify hereditarily altered genes, and the efficient induction of potent immune responses to treat cancer all support the revival of gene therapy (Liu *et al*., 2019)

4.1.6 Gene Therapy in Pancreatic Cancer

PC is a very fatal and challenging form of cancer. Few individuals with pancreatic cancer are candidates for surgery, and traditional chemoradiotherapy is harsh and ineffective. Gene therapy has received a lot of attention as a novel approach to treating pancreatic cancer; it is seen as a fresh and promising approach to caring for PC patients in the future. The P53 tumor inhibitor gene and the mutant K-RAS gene are 2 significant samples in all forms of pancreatic tumors that have been carefully examined and have shown promising outcomes in in vitro and animal models (Xu *et al*., 2018).

The main symptoms of pancreatic cancer are genetic alterations like K-RAS mutations, particularly epigenetic abnormalities of genes linked to a tumor (e.g.), however these have not yet been exploited in clinical studies. The P53 tumor suppressor gene, K-RAS oncogene, VEGFR anti-angiogenic genes, HSK-TK suicide gene, cytosine deaminase and cytochrome p450, and numerous cytokine genes are effective therapeutic targets for gene therapy. When coupled with ifosfamide, a clinical investigation on cytochrome P450 had intriguing outcomes; additional research is being done. VEGFR is the most effective option as a method to prevent angiogenesis. Similar to anti-angiogenesis, clinical trials of the VEGFR-2-targeting deoxynucleotide vaccine and the VEGFR-2-derived peptide vaccination have demonstrated some benefits but not universal efficacy for all patients. They are regarded as a supplemental therapy as a result. The biggest obstacle to the usage and spread of gene therapy is low gene transfer efficiency. Consideration should be given to choosing highly effective vectors that only attack malignant tissues. In general, viral vectors are reported to be used in more than two-thirds of clinical studies because they have longer gene expression and higher transfer efficiency.

Oncolytic viruses have recently demonstrated significant potential in this regard, being able to selectively infect and multiply in malignant cells. Cancerous cells that have been infected by oncolytic viruses can spread to nearby and distant tissues. As a result, intratumoral injection has potential as a treatment for spreading tumors. Oncolytic viruses have been demonstrated to be safe for pancreatic cancer patients when administered systemically, but direct virus injection into the main ulcer is challenging for these individuals (Liu *et al*., 2016)

4.1.7 Breast Cancer

The most typical malignancy in women, breast cancer, has given rise to a number of gene therapy techniques. Neutralization of the mutation, molecular chemotherapy, proapoptotic gene therapy, anti-angiogenesis gene therapy, immunopotentiation (improving the immune response by accelerating and extending its duration), and genetic resistance-sensitivity modulation are a few of them.

The effectiveness, safety, and immunity of gene therapy are being examined in clinical trials for breast cancer. The outcomes of combining radiation or chemotherapy with gene therapy are encouraging. The development of new gene therapy techniques as well as advancements in vector design have all made gene therapy a crucial component of the treatment of breast cancer. TSG P53 is the main target of most clinical trials. The p53 adenoviral vector is best administered intramuscularly. Clinical evidence suggests tumor

relapse in a tiny fraction of individuals despite findings of the transgenic adenoviral gene's high expression. Clinical trial outcomes for gene therapy for breast cancer have so far revealed minimal side effects.

Low clinical response rates and high transgenic gene expression, however, continue to be contentious issues. In order to address this inefficiency, future research must concentrate not only on the new transgenic method but also on the creation of novel gene transfer vectors. Treatment for breast cancer is expected to take a diverse strategy, hybrid therapy, and debulking resections, which reduce tumor size after auxiliary therapies such synchronous or sequential gene therapy, chemotherapy, and radiotherapy (Stoff-Khalili *et al.*, 2019).

4.2. Benefit of Gene Therapy

i. Gene therapy makes use of the powerful immune cells that can be transplanted and the high capacity of stem cell division to remove the treated cells in some circumstances.

ii. iImprovements in the modification of genes and their properties have allowed for the standardization and comparative assessment of the effectiveness of vectors across a range of trials.

iii. The extent and specificity of gene modification in its native or natural position, the integration of vectors in genomic safe sites, the exclusive silence of alleles by synthetic nucleases, and epigenetic alterations offer new prospects for enhancing gene therapy techniques.

iv. Gene therapy makes it possible for biological agents to express themselves continuously, steadily, and on a regular basis by delivering gene cassettes that carry genetic material to specific locations. Cells become intelligent vectors for gene therapy when gene therapy and cell treatment are coupled.

v. Gene therapy directs potent biological processes toward the treatment of disease and the regeneration and repair of tissue.

vi. By conveying information via genetic pathways, the long-term viability and reinforcement of the treatment can be ensured.

5. PROSPECT OF GENE THERAPY

Gene therapy is seen as a significant new method in the third millennium when compared to conventional medicine. By using targeted delivery of gene cassettes carrying genetic material, gene therapy helps biological agents express themselves continuously, steadily, and on a regular basis. Cells become intelligent vectors for gene therapy when gene therapy and cell treatment are coupled. Studies have demonstrated that gene therapy directs potent biological processes toward the treatment of disease and the regeneration and repair of tissues. For instance, by conveying information via genetic pathways, the long-term viability and reinforcing of the administered treatment can be ensured.

Gene therapy makes use of the powerful stem cell capacity and immune cell transplantation to remove the treated cells in some circumstances. Before the full benefits of this therapeutic strategy are realized, further significant obstacles need to be addressed. As an illustration, formulas and cutting-edge engineering designs that incorporate fusing the biological traits of various viruses with synthetic molecules should be used to increase the efficacy and safety of gene transfer vectors. These improvements will relieve cellular restrictions on gene transfer and the passage of sensors through foreign nucleic acids, improve the vector's precision and accuracy in reaching target tissue and cellular models, and increase their ability to carry out targeted. By improving vectors, researchers can also stop the activation of innate and acquired immune systems during gene therapy. In the end, these modifications will guarantee the transplanted gene's high expression and reproducibility over an extended length of time, and the gene expression will occur in a manner comparable to the internal pattern (when the gene is replaced).

The ability to manipulate genes and their properties has advanced, enabling standardization and comparative evaluation of vector performance across a range of trials. The extent and specificity of gene modification in the original or natural position, the incorporation of vectors in genomic safe locations, and the exclusive silencing of alleles by synthetic nucleases and epigenetic modifications all present more opportunities for enhancing gene therapy approaches. The development of novel gene therapy techniques will be aided by a clearer comprehension of the pathology of hereditary, multigenic, or acquired disorders. Long-term care and preventive measures should be taken into account when using gene transfer procedures since they use live biological agents that can have long-term impacts on patients and their sex cells.

Furthermore, there is growing and indisputable worry about the negative impacts of genetic modification due to the poor knowledge of stem cell configurations, tissue regeneration, and immune response assessments. Clinically speaking, improved cell processing and multidisciplinary specialists are sometimes needed for the therapeutic delivery of genes and cell therapy. Additionally, biological readouts should be carried out to keep track of the treatment's security and effectiveness. Pharmaceutical departments and regulatory organizations have given quality criteria for the manufacturing and distribution of these highly specialized medications ever since the first gene therapy developments, from registration to marketing, were made.

In conventional healthcare systems, the complexity and expense of making and delivering live biological medication pose a social challenge to their viability and necessitate the creation of reimbursable policies to ensure that all patients can benefit from them. The ultimate ethical question is whether medical science submits to technological laws or assumes greater accountability for these advancements. Of course, from an ethical standpoint, the creation of methods to alleviate patient suffering justifies our efforts.

6. CONCLUSIONS

The use of DNA (as a drug) to cure disease is known as gene therapy. A gene or genes within cells are also altered as part of an experimental medical procedure to make proteins that alter how those cells function. In gene therapy, the therapeutic protein's DNA is packaged inside a "vector" that is used to deliver it to the body's cells. The cell machinery uses the acquired DNA to express it, which causes the development of a therapeutic protein that addresses the patient's ailments. The foundation of gene therapy is the efficient delivery of the correcting genes, and to do this, researchers have created vectors, which are vehicles for delivering genes.
The therapeutic genes are delivered to the target cells via the vectors. Many of the vectors that are currently in use are built on viruses that have been weakened or altered. Additionally, bacterial circular DNA fragments known as plasmids are employed as vectors. A healthy person's cell is used to extract the therapeutic gene that will be delivered. By utilizing a restriction enzyme to cleave the DNA, the gene is retrieved (restriction enzymes "digest" DNA at designated nucleotide locations along the DNA chain). In gene therapy, numerous methods/protocols are employed. These methods include electroporation, nanoparticles, sonoporation, polymerase chain reaction (PCR), and others.
In the past ten years, clinical gene therapy has achieved a number of advancements. It is possible to name a number of notable achievements, including the medicines that are now accessible for conditions including cystic fibrosis, diabetes, Alzheimer's disease, Parkinson's disease, and other malignancies. In other words, gene therapy encompasses a variety of gene transfer techniques and can be used to treat a wide range of disorders. LV and AAV vectors were initially employed in tests, but new vector systems are anticipated to increase their clinical uses. Lessons learned from the successes, problems, and barriers in recent experiments will guide clinical gene therapy practices toward innovation.
Currently being developed are next-generation procedures that will aid in extending the number of disorders that can be treated with gene therapy. There is minimal possibility of immediate success for some of the most difficult objectives, such as muscular dystrophy and several lysosomal storage and nervous system illnesses. However, continued research will eventually uncover additional therapies. As HSC genome editing has recently demonstrated, gene therapy will be a more precise technique of treatment if it is combined with gene editing tools.

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