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Review Paper

Advancements in Gene therapy: Current and Future of Gene Therapy

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Abstract

Genetic diseases pose serious health challenges globally, arising from mutations in one or more genes. Currently, these disorders lack definitive treatments. Gene therapy is a promising approach that uses genetic material to address the root causes of diseases instead of relying solely on traditional methods like medication and surgery. This technique has shown potential in certain conditions by modifying genetic material, allowing the body to produce necessary proteins or medications. However, gene therapy is not yet available for most diseases and remains largely experimental for many conditions. This review explores the current and future status of gene therapy, focusing on its applications not only for genetic disorders but also for life-threatening diseases such as cancer, cardiovascular disease (CVD), and diabetes. We evaluate the applications and limitations of gene therapy, noting that while rare or inherited genetic disorders may be preventable or curable through targeted interventions, significant challenges remain. Beyond managing blood disorders, retinal diseases, and neuromuscular conditions, gene therapy could potentially eliminate these issues. Moreover, gene-based therapies offer new opportunities for broader therapeutic applications in the future.

Introduction

Genetic diseases are health conditions caused by abnormalities in the genome (Shaath et al., 2024). These abnormalities primarily result from mutations in single or multiple genes or, in some cases, from chromosomal irregularities. While polygenic disorders are the most common, some diseases arise due to mutations in a single gene. The treatment of genetic disorders is

possible through gene therapy, which involves modifying, removing, or replacing defective genes (Shaath et al., 2024; Downie et al., 2024; Brlek et al., 2024).

Gene therapy is an advanced medical approach that enables scientists to treat and prevent diseases by altering genetic material instead of relying on traditional medicine (Taliyan et al., 2024). Healthcare professionals use these techniques to identify the underlying causes of genetic disorders and enhance the patient's ability to produce the necessary proteins and medications (Mascarenhas-Melo et al., 2024). The process involves identifying defective genes, replacing them with normal ones, and transferring the corrected genetic material into the patient's cells. This enables the cells to produce new or modified proteins (Henderson et al., 2024).

The genetic makeup of an organism is found in the nucleus in the form of chromosomes, which consist of DNA and proteins (Dalton et al., 2024). Specific regions of DNA that encode proteins are called coding genes, which contain instructions for protein synthesis (Dalton et al., 2024). Proteins play a crucial structural and functional role in living organisms (Dalton et al., 2024). Sometimes, changes in DNA structure result in altered protein structure and function, leading to genetic abnormalities (Li et al., 2024; Saadi et al., 2024; Huo et al., 2024). Environmental factors can also contribute to these DNA changes, necessitating gene therapy to correct the defective genes (Chang et al., 2024; Caporale, 2024; Fantini et al., 2024).

Advancements in sequencing technology and molecular genetic approaches have revolutionized medical and clinical practices by integrating genetic and molecular medicine into therapy (Brlek et al., 2024; Nikseresht, 2024; Dessai et al., 2024). Gene therapy offers a precise method for treating genetic disorders and has gained significant attention over the last two decades due to its potential to cure diseases (Ataei et al., 2024; He et al., 2024).

Gene Therapy Techniques

Gene therapy involves various genetic materials, including DNA, RNA, small interfering RNA (siRNA), and short hairpin RNA (shRNA) (He et al., 2024; Luo et al., 2024; Verma & Awasthi, 2024). Several advanced gene-editing technologies are employed in gene therapy. CRISPR-Cas9: A revolutionary gene-editing technology that allows precise modifications to DNA. It uses a guide RNA to locate a specific genomic sequence, and the Cas9 enzyme cuts the DNA at that location, enabling the addition, deletion, or alteration of genetic material. TALEN (Transcription Activator-Like Effector Nucleases): Engineered proteins designed to target and cut specific DNA sequences, similar to CRISPR-Cas9, for targeted gene modifications. siRNA

(Small Interfering RNA): Short, double-stranded RNA molecules that can silence specific genes by preventing their expression. They are used in research and potential therapeutic applications to knock down genes.

Gene therapy can be conducted using two main approaches, ex vivo: Procedures performed outside a living organism, typically in a laboratory setting. In this method, patient cells are modified in vitro before being reintroduced into the body. In vivo: Procedures performed within a living organism, where therapeutic genes are directly introduced into the patient's body (Nykänen et al., 2024; Mochi et al., 2024). These techniques have enabled more precise targeting of genetic sequences, improving the efficacy of gene therapy (Kurashina et al., 2024). To transfer genetic material, viral vectors such as adeno-associated viruses (AAV), adenoviruses, lentiviruses, and bacteriophages are commonly used (Kurashina et al., 2024; Słyk et al., 2024). Additionally, non-viral delivery methods, including bacterial vectors, liposomes, and nanoparticles, are also employed (Lim et al., 2024).

Gene Therapy Procedures

Gene therapy works by replacing or modifying defective genes. In some cases, new genes are introduced to treat specific disorders (Guan et al., 2024). This involves inserting a healthy gene into the body, which then replaces the mutated gene (Jeong et al., 2024). The therapy employs various methods, including gene addition, gene silencing, and gene editing.

In Vivo Gene Therapy

In in vivo gene therapy, therapeutic genes are directly delivered into the patient's body. This method introduces new genes to target cells within the body. Gene Addition: This method involves adding new genes into cells. These genes contain instructions for producing essential proteins (Campochiaro et al., 2024). Adeno-associated viruses (AAVs) are commonly used to deliver these genes (Argiro et al., 2024; Kolanu, 2024). Gene Editing: In vivo gene therapy utilizes gene-editing technologies, particularly CRISPR-Cas9, to modify defective genes within the genome. This approach enables precise gene corrections (Kurashina et al., 2024).

Ex Vivo Gene Therapy

In ex vivo gene therapy, patient cells are extracted, modified in the laboratory, and then reintroduced into the body. Gene Addition: Similar to in vivo therapy, this method introduces new genes into cells in a laboratory setting. The cells are then exposed to viral vectors carrying the therapeutic gene. Once modified, the cells are returned to the patient (Campochiaro et al.,

2024). This technique is widely used in stem cell therapy. Gene Silencing: Ex vivo gene therapy can also be employed to silence harmful or faulty genes, preventing their expression. Gene Editing: CRISPR-Cas9 and other gene-editing techniques are used in ex vivo therapy to correct genetic defects in cells before they are reintroduced into the patient (Kurashina et al., 2024). Gene therapy represents a breakthrough in modern medicine, offering a precise and effective way to treat genetic disorders. With continuous advancements in gene-editing technologies and delivery methods, this therapeutic approach holds great promise for the future of personalized medicine.

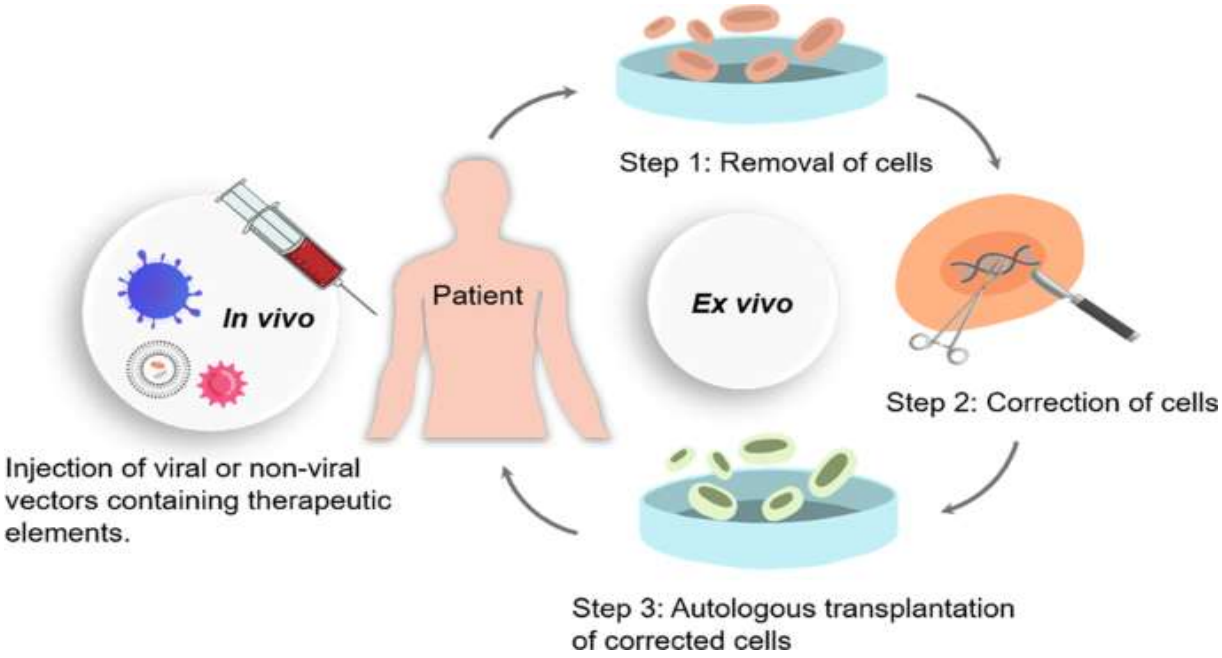

Figure 1. Procedure of gene editing and gene therapy (Li et al., 2020).

Delivery of Genetic Materials

The transfer of genetic material into cells is facilitated by packaging it within a viral vector, which is labeled to ensure it reaches the targeted site (Perera et al., 2024). Viruses are an effective choice for scientists to deliver genetic material into cells due to their natural ability to easily enter cells (Verma and Awasthi, 2024). The harmful genes from the viral genome are removed, allowing the virus to deliver genetic material without causing disease. Genetic material can be transferred into cells or organisms using two primary methods: *in vivo* and *ex vivo*. The *in vivo* method involves the direct delivery of genes into the body, such as through injection, while the *ex vivo* method involves transferring genes into cells outside the body, after which the genetically modified cells are introduced into the body (Bhattacharyya et al., 2024). (Figure 2).

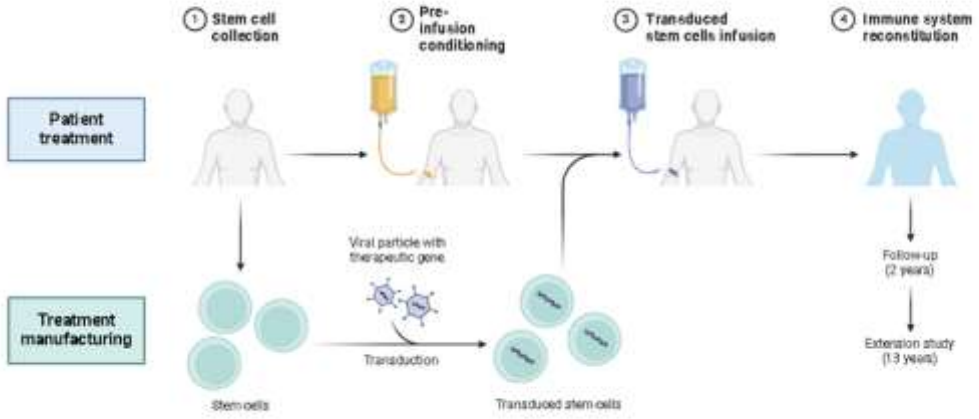

Figure 2. Genetic material delivery to the patient body. Two techniques are used in Vivo and ex vivo. The figure was created in biorender

Current Status of Gene Therapy

Gene therapy remains predominantly in the clinical trial phase, although a number of therapies have received approval from regulatory bodies. The clinical trial phase is essential for evaluating the safety and efficacy of gene therapies (Breda et al., 2023; Abraham et al., 2021; Henderson et al., 2024; Jeong et al., 2024). Currently, gene therapies targeting cancer, degenerative diseases, eye diseases, and infectious diseases such as HIV and HCV are undergoing clinical trials and have demonstrated promising results. Below is a detailed overview of Approved Therapies, Ongoing Trials, and Challenges in Clinical Trials.

Approved Therapies

Several gene therapies have received approval from regulatory agencies, such as the United States Food and Drug Administration (FDA), for the treatment of specific medical conditions. These therapies represent significant advancements in the field of gene therapy and have provided new treatment options for patients with previously untreatable or poorly managed diseases. Below are some examples of approved gene therapies:

Luxturna (Approved in December 2017)

Luxturna (voretigene neparvovec) is a groundbreaking gene therapy approved by the U.S. Food and Drug Administration (FDA) for the treatment of inherited retinal diseases caused by mutations in the RPE65 gene. This condition leads to progressive vision loss and, in many cases, blindness. Luxturna works by delivering a functional copy of the RPE65 gene directly

to the retina using an adeno-associated virus (AAV) vector. This enables the production of the necessary protein to restore visual function, allowing patients to regain partial vision. Clinical trials and real-world applications have demonstrated significant improvements in visual function among patients with retinal degeneration, offering hope to those with previously untreatable genetic blindness (Seoane-Vazquez et al., 2024). Luxturna is particularly notable as it represents the first FDA-approved gene therapy for a genetic disease that causes blindness, marking a major milestone in the field of gene therapy and ophthalmology.

The success of Luxturna has paved the way for further research and development of gene therapies targeting other genetic eye disorders, highlighting the transformative potential of this approach in treating inherited retinal diseases. However, challenges such as high treatment costs, accessibility, and the need for long-term efficacy and safety monitoring remain important considerations.

Zolgensma (Approved in May 2019)

Zolgensma (onasemnogene abeparvovec) is a revolutionary gene therapy developed for the treatment of spinal muscular atrophy (SMA) in children under two years of age. SMA is a severe genetic disorder caused by mutations in the SMN1 gene, leading to the loss of motor neurons, progressive muscle weakness, and, in its most severe form, early death. Zolgensma addresses the root cause of the disease by delivering a functional copy of the SMN1 gene using an adeno-associated virus (AAV) vector. This enables the production of the survival motor neuron (SMN) protein, which is essential for motor neuron function and survival. Clinical trials and real-world applications have shown that Zolgensma can lead to significant clinical improvements, including the recovery of motor function, improved muscle strength, and enhanced survival rates, particularly when administered early in the course of the disease (Chirmule et al., 2024). These outcomes have positioned Zolgensma as a transformative treatment for SMA, offering hope to families affected by this devastating condition.

However, Zolgensma is also one of the most expensive gene therapies globally, with a price tag that has sparked discussions about accessibility and affordability. Despite its high cost, the therapy's potential to provide long-term benefits and dramatically improve the quality of life for children with SMA has solidified its status as a breakthrough in genetic medicine. Zolgensma's success underscores the potential of gene therapy to address previously untreatable genetic disorders, paving the way for further advancements in the field. Figure

3 illustrates the mechanism of action and clinical impact of Zolgensma, highlighting its role in transforming the lives of children with SMA.

Ongoing Trials

Numerous gene therapy trials are ongoing, targeting a variety of diseases. These trials explore both in vivo and ex vivo approaches to correct genetic defects, introduce new genes, and treat a wide range of conditions. Gene therapy trials for cancer involve approaches like immune cell engineering (e.g., CAR-T cell therapy), where patients' T-cells are genetically modified to target and destroy cancer cells. Other strategies include introducing therapeutic genes directly into tumors to induce cell death or improve the immune system's ability to recognize cancer cells.

Gene therapy trials are also being conducted for degenerative diseases like Parkinson's and Alzheimer's disease. These therapies aim to correct genetic defects or introduce genes that can slow or halt the progression of neurodegenerative disorders. In addition to Luxturna, other gene therapy trials are investigating treatments for various inherited retinal diseases, such as Leber's congenital amaurosis and retinitis pigmentosa. These therapies aim to restore or preserve vision by delivering therapeutic genes directly to the retina. Gene therapy is also being explored as a treatment for chronic viral infections like HIV and hepatitis C (HCV). These trials involve modifying immune cells to better fight off infections or delivering genes that can inhibit viral replication (Chirmule et al., 2024).

Challenges in Clinical Trials

While gene therapy offers great promise, there are still significant challenges in clinical trials and the broader development process. As gene therapies are still relatively new, there are concerns regarding their long-term safety. Potential risks include immune reactions to the viral vectors used for gene delivery, unintended genetic modifications, or even the development of secondary diseases, such as cancer, due to gene insertion. Many gene therapies show initial success, but there are concerns about their long-term efficacy. Some therapies may provide temporary relief or improvements but may not offer a permanent cure. The durability of the results is still being studied, and follow-up trials are essential to determine how these treatments perform over time. Gene therapies, especially those that require individualized treatments like Zolgensma, are extremely expensive. This creates a barrier to accessibility, limiting the availability of treatments for those who need them most. The high cost raises questions about the sustainability of gene therapy as a widespread treatment option. The regulatory approval

process for gene therapies is complex and can be slow. While the FDA has approved some therapies, others are still in the trial phase, and approval for new treatments may take years of research and testing. This process needs to balance the speed of access with ensuring patient safety and the effectiveness of treatments (Chirmule et al., 2024).

Figure 3. List of U.S. FDA Approved Cell and Gene Therapy Products *https://bioinformant.com/u-s-fda-approved-cell-and-gene-therapies/*

Current treatment and trials of Gene therapy

Numerous studies have been conducted recently, and some of the recent achievements are discussed here. In July 2021 the researcher at children hospital of Philadelphia have developed a dimmer switch system that control protein expression from gene therapy vector (Xu et al., 2024). The method include alternative RNA splicing using orally available small molecule, and significantly work in all tissue of the body. This method provide significant results to control the protein expressed by gene therapy vector using splicing module (Xu et al., 2024). Researcher at Georgia institute of technology in December 2021 identified novel method in which changes in the genes network associated with cancer were carried out, the results showed significant outcome (Xu et al., 2024). About 90% of changes in gene network in nine types of cancer showed no gene expression (Xu et al., 2024). Hence, the result could be significant for future cancer treatment by changing in the cancer genes the expression of the genes may be stopped. Researcher of American chemical society in December 2021 used CRISPR/cas9 gene editing method, allowing sonodynamic therapy to effectively shrink tumor in mouse model

with liver cancer (Ahmed et al., 2024). Initially, a lipid nanoparticle was constructed containing CRISPR/Cas9 and ROS precursor molecules, which were used on hepatocellular carcinoma cells in a Petri dish. (Ahmed et al., 2024). Basically sonodynamic therapy uses ultrasound and other drugs which release ROS at tumor site but the problem is the cancer cell become more active and show defense against these therapy. Hence, the researcher use CRISPR/cas9 and other ROS molecule with lipid nanoparticle. The results of this therapy in mice model showed significant outcome after 15 days no tumors were observed. Hence this therapy could be significant for the treatment of tumor. Researcher at Brigham and woman hospital in October, 2022 improved the gene therapy delivering to the central nervous system (Qiu et al., 2022). Actually the blood brain barrier is an imposing foe for gene therapy. So the researcher developed vector (Adeno associated virus) system that can cross these blood brain barrier very effectively and deliver the gene to the tissue (Cancer) in brain (Qiu et al., 2022). Hence this therapy could be significant for future gene therapy in brain cancer treatment.

Researcher at University College London in November 2022 invented new gene therapy approach that switch on only in overactive cell and switch off when the activity come to normal (Qiu et al., 2022). This therapy could be significant for neurological and psychiatric diseases, where it could help switch off overactive cells. In May 2023 researcher at University of Barcelona designed new gene therapy methodology to fight against obesity (Riedmayr et al., 2023). Actually ex vivo gene therapy used in mice by transporting manipulated cells, the implanting cell express CPT1AM protein (enzyme) which play vital role in metabolic disorders like diabetics. This therapy could be significant for fighting against obesity and other metabolic disorders.

Researcher in august 2023 at University of Chicago Medical Center used CRISPR/cas9 and edit specific genes in stem cell (Sharma et al., 2023). The building block of blood cell were taken from patients. The therapy were used to edit the genes which increased the expression of fetal hemoglobin protein which replace the unhealthy and sickled hemoglobin in the blood and protect from complication of sickle cell. Patient were treated using its own cell edited with gene therapy, after therapy the patients were observed and results showed decrease in vaso-occlusive events , the painful mechanism occur in sickled RBC which make blockage. Hence this therapy could be significant for future blood disorders. Researcher at University of Zurich in October, 2023 invented a new approach for transferring a large number of genes into cells has been developed, as not all genes are equally transferred through gene therapy (Riedmayr et al., 2023). Now this method is safely transfer all genes into the cell. Researcher at Weill

Advancements in Gene therapy

Cornell medical center in February 2024 identified a set of 140 genes which predict subclasses of lung tumors which are seen to be eliminated by immunotherapies (Dennhag et al., 2024). Hence these genes therapy could be significant for the identification of remaining tumors after immunotherapy and low dose radiation. Researcher from the University of Auckland, Amsterdam University Medical Center and Cambridge university hospital in February 2024 treated 10 patients with CRISPR/cas9 gene therapy (Goyal, 2024). At Umea University researcher in march 2024 found the gene that play key role in eye muscle , the gene fhI2b expressed in eye muscle throughout life but not expressed in other muscle (Jalil et al., 2024). The expression of this gene in zebra fish effected with muscular dystrophy showed significant results by protecting muscles from breakdown. In March 2024 research at UC San Francisco use medicine delivery through amniotic fluid in mice with angelman syndrome a genetic disease (Kochenderfer, 2024). The delivery procedure is more effective as delivering it to the fetal brain via cerebrospinal fluid.

Researcher at University of Helsinki and HUS hospital have succeeded in correcting gene that cause hereditery liver disorder (Clarke et al., 2024). In their recent study they modified the skin cell of patient with ASLD into stem cell. The edited gene in stem cell was carried out using CRISPR/cas9 and the stem cell is differentiated into liver cell (Mueller et al., 2024). Where they observed that the cell no longer produced harmful argininosuccinic acid. So using CRISPR/cas9 gene therapy have significant outcome and corrected the gene without side effect and the cells was also metabolically improved (Clarke et al., 2024). In March, 2024 at University of Zurich Researchers have discovered that a specific mutation in the cancer cells of an aggressive type of blood cancer can prevent novel immunotherapies such as CAR T-cell therapy from working (Fletcher et al., 2024). Their study also explains why the cancer cells are resistant and how this resistance can be overcome: through concomitant pharmacotherapy or genetically improved CAR T-cells.

Successful Gene therapy

Gene therapy has made tremendous progress over the last few decades, with many successful treatments emerging alongside challenges that have shaped current practices. Although gene therapy is not yet widely available, continued research is rapidly progressing toward making it safer, more effective, and accessible to a global patient population (Chanchal et al., 2024). Researchers are working to lower costs, improve treatment safety, and enhance therapeutic outcomes (Li et al., 2024; Sallard et al., 2024). Below, we emphasize key successes like SCID,

beta-thalassemia, and Leber congenital amaurosis (LCA), discuss the failures or risks encountered, and explore the ongoing advancements in the field.

Key Successes in Gene Therapy

Severe Combined Immune Deficiency (SCID)

SCID, also known as "bubble boy disease," is a genetic disorder that results in a severely weakened immune system. One of the earliest successful gene therapy trials targeted SCID. In this approach, stem cells were extracted from the patient's blood, treated with a viral vector to introduce a normal copy of the gene responsible for immune function, and then returned to the patient's body. Initially, the therapy was successful, restoring immune function. However, some trials ended when the viral vector inadvertently activated leukemia in a small number of patients (Kachanov et al., 2024). This unfortunate side effect led to the need for improved viral vectors and more rigorous safety protocols. The SCID trials remain a significant milestone in the history of gene therapy, influencing the development of safer and more efficient treatment vectors.

Beta-Thalassemia

Beta-thalassemia is a blood disorder caused by mutations in the hemoglobin-producing genes. In 2007, a patient with severe beta-thalassemia underwent gene therapy where stem cells were extracted, genetically modified with a retrovirus to correct the defective gene, and then reintroduced into the patient's body. The results showed that the patient's body began producing healthy red blood cells, eliminating the need for regular blood transfusions (Migliavacca et al., 2024). This success demonstrated gene therapy's potential to treat blood disorders and restore normal physiological functions, significantly improving the patient's quality of life.

Leber Congenital Amaurosis (LCA)

LCA is a degenerative eye disorder that leads to blindness due to mutations in the RPE65 gene. In a pioneering gene therapy trial, patients with LCA received an injection of a healthy copy of the RPE65 gene delivered by a viral vector. The results showed significant improvements in vision for up to five years (Sather et al., 2024). However, while the therapy improved vision, it could not stop the ongoing degeneration of the retina. This highlighted the need for

combination therapies to address both the genetic defect and the progressive nature of such conditions.

Gene Therapy for Parkinson's disease

In small trials, gene therapy was used to treat advanced Parkinson's disease by introducing three genes into cells in a small area of the brain using a retroviral vector. These genes allowed cells to begin producing dopamine, a neurotransmitter deficient in Parkinson's patients. The results were promising, with all patients showing improved motor control (Sallard et al., 2024). This approach exemplified how gene therapy could potentially reverse neurological damage in diseases characterized by progressive degeneration.

Gene Therapy for Lipoprotein Lipase (LPL) Deficiency

In patients with LPL deficiency, gene therapy using an adeno-associated virus to deliver a working copy of the LPL gene has been successful. LPL is a protein that helps break down fats in the blood, and without it, toxic fat buildup occurs. The gene therapy has shown improvement in the ability of muscle cells to metabolize fat, preventing complications (Yuan et al., 2024).

Failures and Risks in Gene Therapy

While gene therapy has shown great promise, it has not been without its setbacks, highlighting the risks that need to be addressed.

Leukemia Activation in SCID Trials

The activation of leukemia in some SCID patients due to the viral vector was one of the most notable failures. The retroviral vectors used to deliver the therapeutic genes inadvertently integrated into the genome in a harmful way, leading to the activation of oncogenes. This risk has led to a significant shift toward the development of safer vectors that are less likely to integrate into critical parts of the genome, minimizing the chance of activating harmful genetic mutations (Kachanov et al., 2024).

Challenges with Long-Term Efficacy in LCA

Although gene therapy for LCA improved vision for some patients, it was not able to halt the ongoing degeneration of the retina. This underscores the limitation of gene therapy in treating

degenerative conditions where the underlying disease mechanism may require continuous intervention or combination therapies to prevent further damage (Sather et al., 2024).

Ongoing Advancements and Future Directions

Despite the challenges, researchers continue to make significant advancements in gene therapy.

Improved Vector Systems

The search for safer and more effective vectors continues. Current research is focused on improving viral vectors, reducing their immunogenicity, and ensuring that they deliver the therapeutic genes without causing harm or unintended genetic alterations (Wilson et al., 2024). Non-viral delivery methods are also being explored to enhance safety.

Safer CRISPR Gene Editing Techniques

CRISPR/Cas9 technology has revolutionized gene editing, offering a way to precisely modify genes in vivo. However, concerns over off-target effects and the potential for unintended genetic alterations remain. Researchers are working on refining CRISPR technology to make it safer and more precise, with the goal of minimizing these risks while increasing therapeutic efficacy (Sallard et al., 2024).

Cost Reduction and Accessibility

The high cost of gene therapy remains a significant barrier to widespread access. Efforts are underway to reduce the cost of gene therapies, making them more accessible to patients around the world. Researchers are exploring more affordable manufacturing processes and ways to streamline clinical trials to make these therapies more cost-effective for both patients and healthcare systems (Li et al., 2024).

Conclusion and Future of Gene Therapy

It can be concluded that gene therapy holds great promise as a new hope for better treatment and control of life-threatening diseases. Furthermore, gene therapy is crucial for studying the nature and underlying causes of diseases, enabling earlier treatments, personalized medicine, and targeting the specific genes responsible for the disease. Research is ongoing, with scientists around the world working to develop more effective and safe gene therapy approaches to treat and potentially cure various disorders.

Gene therapy has diverse applications, although there are limitations. The field is still in its early stages, and there are no guarantees of a cure or significant outcomes. There may be potential side effects, no guarantees of treatment success, and risks such as autoimmune responses from the patient's body. Additionally, the long-term effects of gene therapy remain unknown. Despite these challenges, we conclude that more clinical trials are needed, and such trials are ongoing. In the near future, researchers are expected to develop more efficient and significant gene therapy approaches. In the future, gene therapy is likely to become a major treatment option for rare, genetic, and life-threatening diseases. The current status of gene therapy shows that it is still primarily in the early stages of trials, and no definitive, widespread success has been achieved. While some small-scale studies have shown promising results, larger and more extensive research is needed. Nonetheless, gene therapy is emerging as a hopeful solution for treating genetic disorders and other diseases.

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