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A Comprehensive Exploration of Gene Therapy and its Transformative Applications

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ABSTRACT

At the vanguard of medical discovery, gene therapy offers a revolutionary approach to treating chronic diseases and hereditary problems. Delves into the complex field of gene therapy, this in-depth examination reveals its workings, obstacles, and innovative uses. The review takes readers on a journey through the development of gene therapy, from its theoretical beginnings to the state-of-the-art technologies that have shaped its present. A variety of gene delivery techniques, including viral vectors and CRISPR-Cas9, are discussed, along with their advantages and disadvantages. The paper also clarifies the implications for personalized treatment and the ethical issues underlying gene editing. Case studies and success stories demonstrate the real-world effects of gene therapy on illnesses ranging from hereditary problems to specific cancer kinds. As a result, this review presents a comprehensive understanding of gene therapy by combining clinical successes, scientific discoveries, and ethical issues. It provides a comprehensive overview of the present and future directions of gene therapy as well as its significant influence on the field of modern medicine, making it an invaluable tool for researchers, practitioners, and enthusiasts alike.

***Keywords**: Gene therapy; ADASCID, Cystic fibrosis, History*

INTRODUCTION

Targeting and altering the underlying genetic material within cells directly through gene therapy is a novel way to treating genetic abnormalities and other diseases. To rectify or lessen the impact of genetic mutations, the basic idea is adding, fixing, or replacing certain genes. This treatment plan makes use of the tools and technologies available to genetic engineers to treat a variety of diseases, from inherited genetic problems to specific cancers. With the conception of the Human Genome Project and the subsequent deciphering of the human genome, which gave scientists a detailed map of our genetic composition, the journey toward gene therapy was commenced. Gene therapy has become increasingly important in biomedical research and clinical applications as a result of developments in molecular biology, virology, and gene-editing methods like CRISPR-Cas9. Typically, gene therapy techniques entail introducing therapeutic genes into a patient's cells by non-viral or viral vectors. Target cells integrate or express the corrected genetic information when the

therapeutic genes are delivered into them using viral vectors, which are frequently modified viruses. Therapeutic DNA or RNA can be directly injected as a non-viral approach. Gene therapy is showing amazing results in some situations as research and clinical trials move forward, giving patients with genetic illnesses hope and opening the door to personalized care. A major turning point in the continuous search for novel and efficient therapeutic approaches could be reached with the development of gene therapy, which has the potential to revolutionize the way we approach and treat a broad range of medical problems.

History of gene therapy

1. **Early Theoretical Concepts (1960s-1970s):**
   The concept of therapeutic gene manipulation was initially put forth in the 1960s. Researchers like Paul Berg and Joshua Lederberg talked about the potential use of genetic engineering to treat hereditary diseases.[1]
2. **First Attempted Gene Transfer (1970s-1980s):**
   In an effort to treat genetic problems by introducing genes into cells, researchers made the first attempt at gene therapy in the 1970s and early 1980s. However, the field was still in its infancy and these early initiatives encountered difficulties.[1]
3. **Discovery of Viral Vectors (1980s):**
   Researchers started looking into using viral vectors to deliver therapeutic genes in the 1980s. The capacity of several viruses, including adenoviruses and retroviruses, to transfer genes into target cells has been studied.[1]
4. **First Clinical Trials (1990s):**
   The first gene therapy clinical trials were started in the 1990s. The first licensed clinical trial for gene therapy was conducted in 1990, specifically for a patient with a rare immunological condition called adenosine deaminase (ADA) deficiency.[1]
5. **Setbacks and Safety Concerns (1990s-2000s):**
   There were challenges in the field in the late 1990s and early 2000s. Concerns over safety were raised and gene therapy research came under closer examination after a patient named Jesse Gelsinger passed away in a clinical experiment in 1999.
6. **Advancements in Viral Vectors and Techniques (2000s-Present):**
   Gene therapy has become much more accurate and effective over time thanks to developments in gene-editing methods like CRISPR-Cas9 and viral vector design.
7. **success Stories and Regulatory Approvals (2010s-Present):**
   Several successful gene therapy trials and regulatory approvals occurred in the 2010s. The profession saw a sea change when therapies for inherited retinal diseases, spinal muscular atrophy, and specific forms of leukemia were approved by regulators.[1]

Importance

In the 1980s, there was a lot of business interest in gene therapy. This occurred, in part, because many believed that the proof of concept to clinical trials for such a medicine would happen quickly and smoothly. But those optimism were short-lived as the first patient in a gene therapy study passed away in 1999. It would be ten more years before there was any renewed hope for the therapy. Gene therapy became the focus of dozens of new start-ups starting in 2008. Pharmaceutical corporations and the stock market provided sponsorship for these to be established. The stock market's appraisal of Juno Therapeutics reveals just how much importance gene therapy started to receive. 2014, an only year following Juno's establishment, the business The business was valued at $4 billion USD. Eighty-five businesses were developing gene treatments when the first one was approved in the United States. By the end of 2020, there were 1085 businesses operating in that field, with over 400 gene therapy trials underway, according to the Alliance for Regenerative Medicine.[2]

Discovery of gene therapy

In the late 1960s, researchers showed for the first time that it was possible to add new genetic functions to mammalian cells. There were several approaches taken. One entailed directly introducing genes into a living mammalian cell using a micropipette. One more exposed cells to a precipitate of desired gene-containing DNA. Another option for introducing the genes into cells would be to utilize a virus as a vector or vehicle.[3]

Lorraine Kraus of the University of Tennessee was among the first to report the direct insertion of functioning DNA into a mammalian cell. She was able to genetically modify the haemoglobin of bone marrow cells from a sickle-cell anemia patient in 1961. She achieved this by culturing the patient's cells using DNA taken from a donor who had normal hemoglobin. After seven years, Lesch-Nyhan syndrome, a crippling neurological condition, was successfully linked to genetic flaws that were effectively rectified by Theodore Friedmann, Jay Seegmiller, and John Subak-Sharpe at the National Institutes of Health (NIH), Bethesda. They achieved this by introducing foreign DNA into cultured cells taken from disease-stricken people.[3]

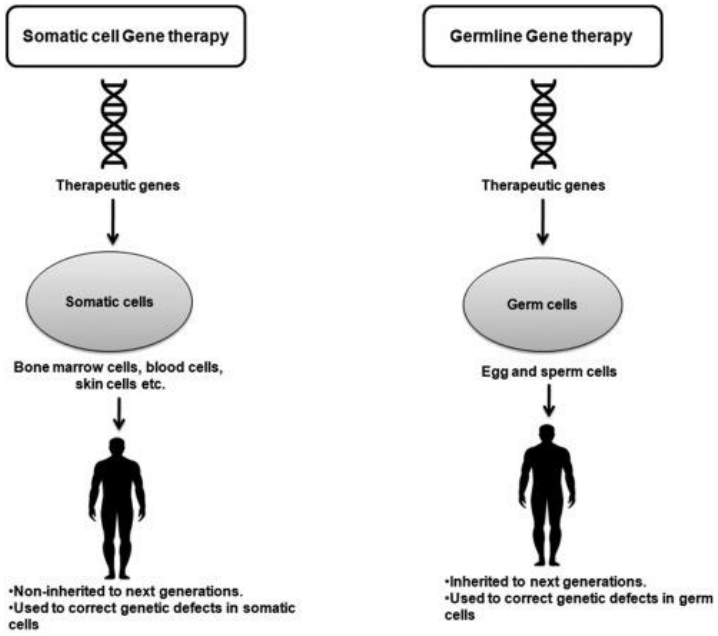

Figure. 1 somatic and germline gene therapy[11]

In 1970, the first human recipients of gene therapy were treated. Two very young West German sisters with hyperargininemia—a very rare genetic condition that inhibits the formation of arginase—were given it. This enzyme aids in preventing the accumulation of arginine in body fluids. Any buildup can result in neurological and muscle issues, seizures, and brain damage. A rabbit virus called Shope papilloma, which is known to stimulate the synthesis of arginase, was injected into each sister. The shot was administered as a final, last-ditch effort to save the kids. American physician Stanfield Rogers and German pediatrician H. G. Terheggen administered the treatment. Based on observations Rogers had previously made on laboratory technicians at Oak Ridge National Laboratory who contracted the rabbit virus while handling it, they decided to take the chance. The technicians had abnormally low blood levels of arginine but no negative effects from the infection. Even in a technician who had not seen the virus in twenty years, this was evident. Rogers linked a gene in the rabbit virus that was known to promote the synthesis of arginase in rabbits to the aberrant arginine levels observed in the technicians. Rogers anticipated that by injecting the girls with the rabbit virus, their cells would acquire the genetic instructions necessary to manufacture arginase. Following the treatment of the two sisters, a third sister was born with hyperargininemia. The virus was injected into her as well. Sadly, none of the sisters showed any improvement during the therapy.[4]

The discovery of genetic engineering in the early 1970s created a new avenue for gene therapy. The method offered two important resources. a method for cloning particular disease genes, to start. Second, a successful gene-transmission technique. The American scientists Richard Roblin and Theodore Friedmann were the first to draw attention to the technology's potential for gene therapy. A 1972 Science article proposed the use of genetically engineered tumor viruses to transfer genetic information needed to treat people with hereditary diseases.

The first application of the technology was in the treatment of beta-thalassemia. This blood condition, which is linked to a hereditary flaw in the beta-globin gene, typically results in early mortality. In 1976, researchers at Harvard University and Cold Spring Harbor Laboratory successfully cloned the beta-globin gene for the first time. That was the first cloned disease gene ever. Three years later, the effective insertion of the gene into the bone marrow of irradiated mice was reported by a team at the University of California, Los Angeles, headed by Martin Cline. After that, Cline and his colleagues attempted in vain to treat two individuals with beta-thalassemia, one in Israel and the other in Italy, by reintroducing the gene into their bone marrow after it had been removed. Cline received harsh criticism right away for not getting approval from the Institutional Review Board at his home institution to conduct the research and for not having enough animal data to support the efficacy of his method. Cline lost both his university chair and the majority of his NIH money as a

result of the event. Additionally, it sparked a heated public discussion on the moral and social ramifications of gene therapy. As a result, guidelines for further human gene therapy testing were strengthened and placed within the purview of the NIH's Recombinant DNA Advisory Committee (RAC).[4]

The discovery of retroviruses, a far more effective method of gene transfer, in the 1980s ushered in a new age for gene therapy. Richard Mulligan, a researcher at Massachusetts Institute of Technology and a former PhD student of Paul Berg, a significant figure in the history of genetic engineering at Stanford University, created the first viable retroviral vector for use in gene therapy. Together with his colleagues, Mulligan had genetically altered a mouse leukemia retrovirus by 1983 so that it could carry any desired DNA without proliferating in people. Additionally, the new vector had a selective marker—a fragment of Escherichia coli bacteria's DNA—that allowed one to determine the number of genes a cell acquired during gene transfer.[5]

French Anderson, a geneticist at the National Heart, Lung, and Blood Institute of the National Institutes of Health, was among the first to deploy Mulligan's novel vector. By 1989, he had obtained approval from the RAC to start the first gene therapy clinical trial that was authorized. The pediatrician and immunologist Michael Blease was to assist with this. Children with severe mixed immunodeficiency, a hereditary immunological condition brought on by a faulty adenosine deaminase (ADA) gene, were the target population for the team's gene therapy experiment. The condition's majority of newborns did not have long lives and were only able to survive by being kept in sterile plastic cages, which is how the phrase "bubble disease" came to be. There were just two alternatives for treating those who had the illness. The first involved receiving a bone marrow transplant, but this was complicated by the requirement to locate a compatible donor and the potential for an adverse reaction. The second involved receiving regular injections of the synthetic enzyme PEG-ADA. After the initial injection, children receiving this kind of treatment typically showed a noticeable improvement; however, this improvement was typically transient, and following doses were essentially ineffectual.[5]

Early in 1990, Anderson's team began testing gene therapy in children with ADA-SCID. The four-year-old Ashanti DeSilva was the first patient to get the therapy. Twelve days were spent on her treatment. Ashanti's blood cells had to be removed, a fresh copy of the ADA gene had to be inserted into them, and the cells had to be reinfused into her. The process was comparable to a bone marrow transplant overall. Resupplying Ashanti's blood with cells capable of producing ADA was the aim. The benefit of gene therapy was that there was no risk of rejection because the cells came from Ashanti. Ashanti improved to such an extent that she could no longer be kept in isolation and could begin school, much to everyone's delight.[6]

In the 1990s, many gene therapy trials were started in response to Ashanti's success. During this decade, a big change occurred. Crucially, the profession shifted away from focusing only on treating uncommon illnesses brought on by a particular gene, as Ashanti had been. By the year 2000, almost 3,000 patients have participated in nearly 400 trials testing gene therapy. The majority of the trials focused on cancer, although they also looked into Gaucher disease, AIDS, cardiovascular illness, and cystic fibrosis.[6]

In 2003, China granted a license for the first gene treatment. This medication, which was created to treat head and neck cancer, was not exported to other nations. Nine years later, Europe approved the first gene therapy. It was created by the Dutch business UniQure to treat lipoprotein lipase insufficiency, a rare metabolic illness that results in pancreatic inflammation and both acute and chronic stomach pain. However, because not enough patients need the medication, it was not a commercial success. As a result, by 2017, UniQure had withdrawn the drug's marketing authorization.

A second gene treatment developed by GlaxoSmithKline for children with ADA-SCID was licensed in Europe in 2016. After a year, Novartis was able to get the first gene therapy approved in the US. The therapy, which was created to treat acute lymphoblastic leukemia, originated from the first research done in 1989 by Anderson and Rosenberg to demonstrate the safety of gene therapy for treating children with ADA-SCID.[7]

Types of gene therapy

Somatic gene therapy

In human somatic cells, this kind typically manifests itself. The sole person affected by the damaged cells will get new, healthy cells added to them. This is specific to that person. The human body's stem cells or somatic cells are infused with therapeutic genes using this technique. It is thought that this approach of gene therapy is the most effective and secure. Many diseases, including muscular dystrophy, cancer, cystic fibrosis, and some infectious diseases, can be treated by somatic cell gene therapy.[8,9]

Germlline gene therapy

It takes place in the body's germline cells. This approach is typically used to correct genetic mutations in genes that cause disease and are inherited by a child's parents. The procedure entails grafting a healthy DNA into the cells that make sperm, eggs, or other reproductive cells. Since the risks of germline gene therapy outweigh the benefits, it is illegal in many states.[8][9]

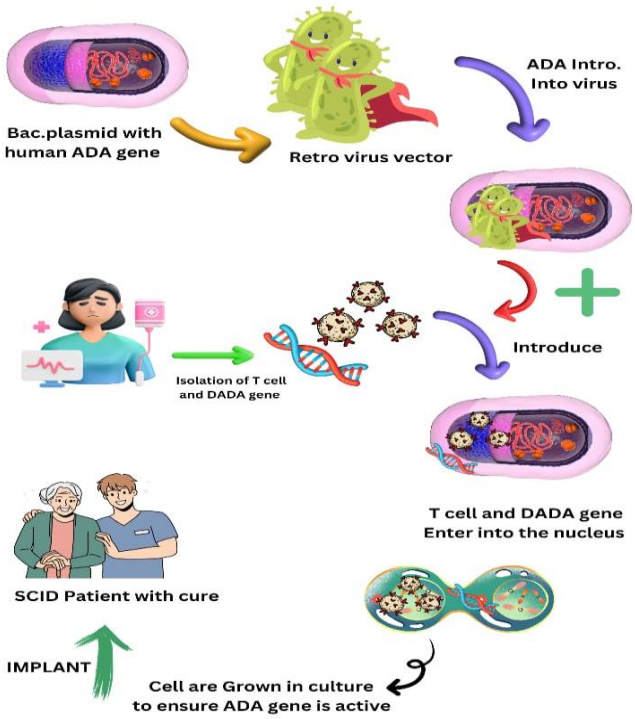

Figure 2. ADASCID

Although human medical intervention involving gene modification is still in its infancy, examples of this type of modification already exist. These include cows that have been genetically altered to produce more milk or to be able to secrete human hormones, as well as "knockin" and "knockout" mouse models that have been used for decades to study the function of specific genes.[10]

Approaches of gene therapy

1. **Treatment using Gene Augmentation:**
- The goal is to introduce a working copy of a missing or damaged gene.
- Method: Using viral vectors or other delivery mechanisms, a functional copy of the gene is inserted into the patient's cells.[12]

2. **Gene Inhibition Treatment:**
- The goal is to prevent a dangerous gene from being expressed as much as possible.
- Method: Specific genes can be silenced or had their expression inhibited by using strategies like antisense oligonucleotides or RNA interference (RNAi).[12]

3. **Therapy Using Gene Editing:**
- The patient's DNA will be directly altered in order to replace or fix a defective gene.
- Method: Within the patient's cells, particular DNA sequences are targeted and edited using technologies such as CRISPR-Cas9.[12]

4. **Gene therapy using cells:**
- The aim is to alter or work on cells outside the body prior to grafting them into the patient.
- Procedure: The patient or a donor's cells are taken out, genetically altered ex vivo (outside the body), and then re-implanted into the patient.[12]

5. **Viral Vector Distribution:**
- The goal is to introduce therapeutic genes into target cells by using modified viruses as carriers, or vectors.
- Method: To transport the repaired genetic material to target cells, viruses, such as lentiviruses or adenoviruses, are designed to carry the therapeutic gene**.**

6. **Non-Viral Vector Transmission:**
- Purpose: To use non-viral methods for gene delivery.
- Method: Without using viral vectors, genetic material can be introduced by methods like as lipofection, electroporation, or direct injection of naked DNA**.**[13]

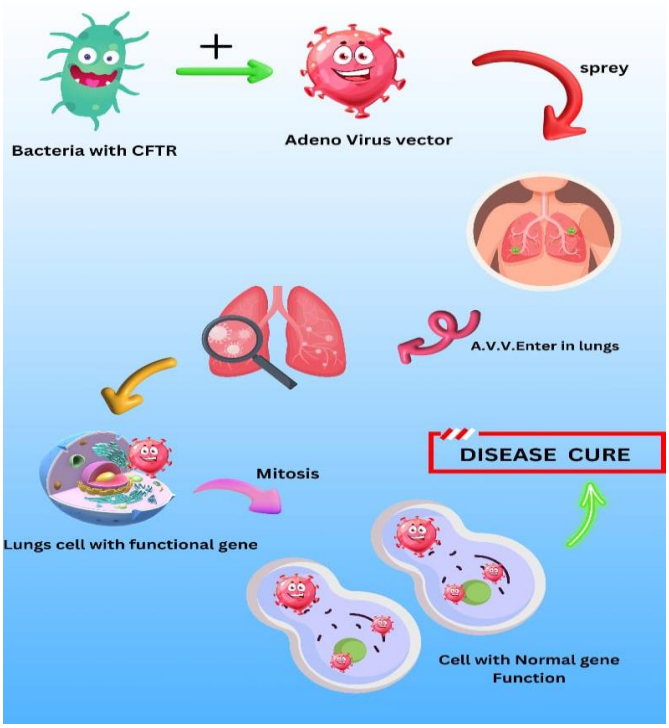

Figure. 3. Cyctic fibrosis

- **Gene therapy in vivo:** The goal is to directly implant the therapeutic genes into the patient's body.
- Technique: The patient's target tissue or organ receives gene therapy directly.

7. Ex vivo Genetic Counseling:

- The goal is to alter cells that are not within the body before reintroducing them into the patient.
- Method: The patient has their cells extracted, genetically altered outside of the body, and then reinfused.[13]

ADASCID

A uncommon genetic condition known as ADA-deficient severe combined immunodeficiency (ADA-SCID) is defined by a lack of the ADA enzyme, which is essential for a healthy immune system. As a result, the immune system is significantly weakened, making the afflicted people more vulnerable to diseases. In order to treat ADA-SCID, a functional ADA gene is usually inserted using a viral vector into the patient's hematopoietic stem cells. After that, the patient receives a second transplant of the genetically altered cells in an effort to trigger a healthy immune response. This novel technique has possibilities for treating this potentially fatal illness in a way that could be curative.[14]

Procedure of adascid gene therapy

1. Evaluation of the patient and diagnosis:

- Confirm the diagnosis of ADA-SCID using genetic testing and other diagnostic procedures.
- Assess whether the patient is a good candidate for gene therapy and for general health[15].

2. Gathering of Patient Cells:

- Hematopoietic stem cells (HSCs) are often extracted from the patient's peripheral blood or bone marrow.

3. Gene Transmission:

- Utilizing a viral vector, introduce a functioning ADA gene into the patient's HSCs. Modified viruses known as viral vectors are able to introduce the therapeutic gene into the patient's cells.
- Usually, lentiviruses or retroviruses are used as the viral vector in ADA-SCID gene therapy.[16]

4. Culture and Growth of Cells:

- To get enough genetically altered cells for transplantation, cultivate and multiply the cells in the lab.[16]

5. Exercise Program:

- For the genetically altered cells to engraft, the patient may need to go through a conditioning

program that includes radiation therapy or chemotherapy.[17]

6. **Replantation:**
- Reintroduce the genetically altered HSCs into the patient's circulation. The cells will migrate to the bone marrow and begin creating functioning immune cells with the corrected ADA gene.[18]

7. **Observation and Succession:**
- After transplant, keep an eye on the patient's general health and immune system recuperation.
- Check immune system and ADA enzyme levels on a regular basis.[18]

8. **Extended Follow-Up:**
- Monitoring the gene therapy's long-term safety and efficacy requires ongoing observation.

cystic fibrosis

A genetic condition that affects the reproductive, digestive, and respiratory systems is called cystic fibrosis (CF). It is brought on by mutations in the CFTR gene and affects the pancreatic ducts and airways by producing thick, sticky mucus. Nutritional inadequacies, stomach problems, and recurrent lung infections are caused by this mucus accumulation. A chronic cough, lung infections, dyspnea, and stunted growth are among the symptoms. Although there is no known cure for cystic fibrosis (CF), treatment methods include medication management, physical therapy, and dietary support to manage symptoms. The goal of new discoveries in CF research and treatments, such CFTR modulators, is to enhance the lives of those who have CF.[19]

Procedure of cyctic fibrosis gene therapy

1. **Finding Genetic Mutations:**
- Identify the patient's particular CFTR gene mutation.

2. **Vector of Delivery (Viral):**
- Introduce a functioning CFTR gene into the afflicted cells using a viral vector, most commonly an adenovirus or an adeno-associated virus.[20]

3. **Getting the Gene Therapy Vector Ready:**
- In the lab, insert the active CFTR gene into the viral vector.

4. **Management of the Airways:**
- Deliver the gene therapy vector to the patient's airways, commonly through inhalation.

5. **Target Cell Uptake:**
- Permit the target cells—usually the respiratory tract's lining cells—to absorb the altered viral vector.

6. **Production of a Working CFTR Protein:**
- A functional CFTR protein is produced after the functional CFTR gene is expressed inside the cells.

7. **Adjustment of Ion Transport:**
- The lungs, in particular, operate better when the CFTR protein is functioning because it corrects the faulty chloride ion transport across cell membranes, lowering mucus thickness.[21]

8. **Observation and Succession:**
- Keep an eye on the patient's general health, improvement in lung function, and decrease in respiratory symptoms.

CONCLUSION

This review presents a comprehensive understanding of gene therapy by combining clinical successes, scientific discoveries, and ethical issues. It provides a comprehensive overview of the present and future directions of gene therapy as well as its significant influence on the field of modern medicine, making it an invaluable tool for researchers, practitioners, and enthusiasts alike.

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Conflict of interest

The author declares that there isn't any conflict of interest regarding the publication of this paper.

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