

REDEFINING MEDICINE: GENE THERAPY AS A NOVEL THERAPEUTIC STRATEGY

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Abstract- Gene therapy is a unique approach to treat or prevent many diseases by utilizing genes from multiple sources. By introducing a gene into a patient's cells, an illness (including hereditary disorders, some forms of cancer, and specific viral infections) can be treated without the need for medication or surgery. This technique's basic idea is to replace, inactivate, or introduce a new gene into the body for purposes such as gene augmentation, metabolic modification, or surgical methods. In order to insert a new gene, gene therapy employs a variety of techniques. These include the use of viral vectors (such as retroviruses and adenoviruses), non-viral vectors (such as injections of naked DNA), physical methods (such as electroporation and sonoporation) or chemical methods (such as oligonucleotides and hybrid methods) to enhance gene delivery. This novel method presents a number of benefits and drawbacks in addition to numerous ethical and social issues that make its practical implementation challenging. In conclusion, researchers think that gene therapy represents the most promising approach to treating various illnesses. In the realm of medicine, gene therapy is exploding; specialists predict that in 20 years, this will be the final treatment for all hereditary diseases.

Keywords: gene therapy, gene delivery, viral & non-viral vectors, pros and cons.

INTRODUCTIONS

Gene is a structural, functional and mutational unit of DNA. Change in natural coding property of a gene is called mutation which is often lethal. Correcting that mutation is called gene therapy. This is a technique whereby the absent or faulty gene is replaced by a working gene, so that the body can make the correct enzyme or protein and consequently eliminate the root cause of the disease. The treatment of gene therapy involves researchers replacing the defective or faulty genes with a normal functioning gene. It involves detection of gene, determination of its role, cloning and introducing the gene by proper way. This is either germ line gene therapy (done in germ cells) or somatic gene therapy done in somatic cells¹.

Technological advances and the ever growing knowledge of molecular virology and virus-host cell relationships have constantly improved the safety profile of viral vectors that are now used in vitro and in vivo to study cellular gene function, to correct genetic defects (gene therapy), express therapeutic proteins, vaccinate against infectious agents and tumors, produce experimental animal models, and for other purposes. One of the main focuses of this technique is the optimization of delivery vehicles (vectors) that are mostly plasmids, nano structured or viruses. The viruses are more often investigated due to their excellence of invading cells and inserting their genetic material. While originally conceived as a way to treat life threatening disorders (inborn errors, cancers) refractory to conventional treatment, gene therapy now is considered for many nonlife threatening conditions, including those adversely affecting a patient's quality of life. The lack of suitable treatment has become a rational basis for extending the scope of gene therapy. The potential therapeutic applications of gene therapy are vast. A major advantage of gene therapy over the use of conventional drugs is the prospect of curing disease rather than providing transient relief by suppression of disease symptoms. Replacing defective genes with functional genes through the use of gene therapy offers the prospect for long term therapeutic benefits without repeated drug application².

The objectives of this seminar are:

- 1) To give an overview on types and methods of gene administration.
- 2) To give highlight on current applications and future perspective of gene therapy.
- 3) To summarize challenges toward application of gene therapy.
- 4) To overview viral and physical agent used as gene transfer vehicle or vector³.

HISTORY OF GENE THERAPIES

Human gene therapy has undergone rapid development since Theodore Friedmann and Richard Roblin published the first accounts on its potential and challenges in 1972. Since then, scientists have strived to develop a safe, long-lasting,

and effective method for modifying and manipulating human cells to achieve therapeutic effects for various heritable diseases. In 1989, the first approved human gene therapy trial took place, using a retroviral vector to insert a gene encoding neomycin resistance into human tumor infiltrating lymphocytes for the treatment of metastatic melanoma. Just under a year later, another group of researchers conducted the first approved gene therapy trial for heritable genetic diseases⁴.

In that study, a retroviral vector delivered a functional copy of the adenosine deaminase gene to explanted T lymphocytes obtained from patients with adenosine deaminase deficient severe combined immune deficiency. Despite these landmark studies, the death of 18-year-old Jesse Gelsinger in 1999 highlighted the dangers of gene therapy at the time. Mr. Gelsinger had partial ornithine transcarbamylase deficiency and participated in a trial aiming to use adenoviral vectors to deliver functional ornithine transcarbamylase genes. More than 3.8×10^{13} adenoviral particles were injected into his liver, triggering a cytokine storm and acute respiratory distress leading to his death⁵.

In 2020, the FDA released 6 guidelines, standardizing and streamlining the drug approval process for new gene therapies. In response to the SARS-CoV-2 pandemic, gene therapy achieved a major breakthrough with the approval and adoption of 2 messenger RNA (mRNA)-based vaccines. These vaccines used novel lipoprotein nanoparticles to safely deliver the genetic payload into the cell cytoplasm. Currently, 27 approved gene therapies are available in the United States with dozens of clinical trials underway⁶.

In the field of cardiology, some pioneering studies of gene therapy were first performed in 1990, in which a murine retrovirus was used to deliver a recombinant beta-galactosidase gene into the wall of an arterial segment. In 1996, Dr Jeffrey Isner used a plasmid containing vascular endothelial growth factors, which was suspended in a hydrogel polymer and coated onto an angioplasty balloon that was applied to the distal popliteal artery to induce angiogenesis. This approach laid the foundation for a series of clinical trials aimed at injecting naked plasmid DNA encoding for angiogenic factors like vascular endothelial growth factor into or at the walls of coronary and peripheral arteries, as well as into the myocardium itself. However, none of these trials had a significant impact on clinical outcomes. In 2007, the first gene therapy clinical trial for patients with heart failure aimed to determine the effects of delivering a gene expressing SERCA2a through an AAV to improve calcium handling within cardiomyocytes where the Ca^{2+} ATP-ase was down regulated. Unfortunately, the treatment failed to improve clinical outcomes⁷.

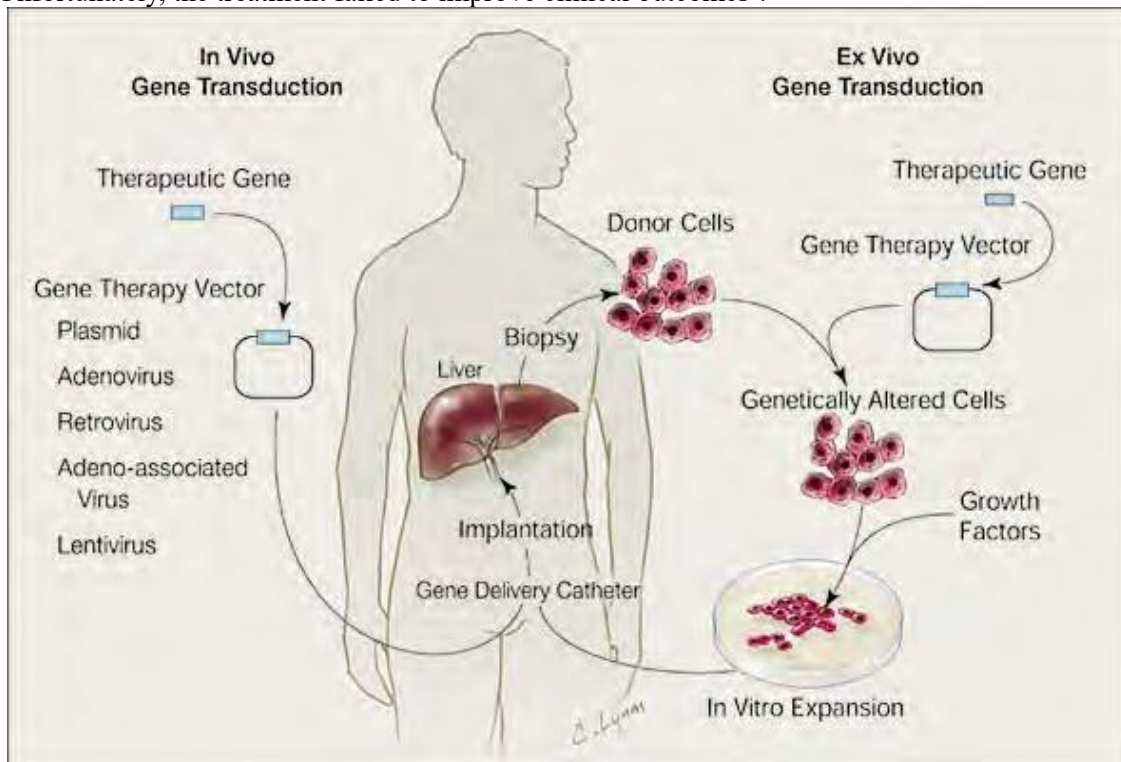


Fig No. 1

Approaches for gene therapy There are two approaches to achieve gene therapy:

1) Somatic gene therapy- It involves the insertion of a functional and expressible gene into a target somatic cell to correct a genetic disease. It represents the mainstream line of current basic and clinical research where any modifications and effects will not be inherited by the patient's offspring or later generations. Somatic gene therapy is viewed as a more conservative and safer approach because it affects only the targeted cells in the patient and is not passed on to future generations; however, somatic cell therapy is short lived because the cells of most tissues ultimately die and are

replaced by new cells. In addition, transporting the gene to the target cells or tissue is also problematic. Regardless of these difficulties, however, somatic cell gene therapy is appropriate and acceptable for many disorders⁸.

Several key steps appear to be involved in effective gene transfer to somatic cells:

- Type of delivery vehicle that may be composed of cationic liposomes, other types of liposomes, polymers, and their combinations, various types of viral or hybrid vectors and combinations of viral vectors with polymers or lipids.
- Interaction of the gene vehicle with serum components.
- Its circulation time in body fluids and bio distribution⁹.

2) Germline gene therapy- In this approach, functional genes are introduced into germ cells (sperm or egg). Therefore the changes due to therapy would be heritable. Although this approach is highly effective in counteracting genetic and hereditary diseases, but for safety, ethical and technical reasons, germline gene therapy is not being attempted at present. The genetic alterations in somatic cells are not carried to the next generations. Therefore, somatic gene therapy is preferred and extensively studied with an ultimate objective of correcting human diseases¹⁰.

Types of gene therapy

There are two types of gene therapy as described.

1) Ex vivo gene therapy: This technique involves the following steps

a) Isolate cells with genetic defect from a patient b) Grow the cells in culture c) Introduce the therapeutic gene to correct gene defect d) Select the genetically corrected cells and grow e) Transplant the modified cells to the patient The procedure basically involves the use of the patient's own cells for culture and genetic correction, and then their return back to the patient.

2) In vivo gene therapy: The direct delivery of the therapeutic gene into the target cells of a particular tissue constitutes in vivo gene therapy. Many tissues are the potential candidates for this approach. For example liver, muscle, skin, spleen, lung, brain and blood cells etc.

The success of in vivo gene therapy mostly depends on the following parameters:

- The efficiency of the uptake of the therapeutic gene by the target cells
- Intracellular degradation of the gene and its uptake by nucleus
- The expression capability of the gene.
- Its interaction with the surface of the cell.
- Its triggering of apoptotic pathways by this interaction.
- Its penetration through the cell membrane barrier.
- Its release from endosomes or other sub cellular compartments and its escape from degradation by intracellular nucleases.
- Nuclear import¹¹.

Disease	Gene therapy
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA)
Cystic fibrosis	Cystic fibrosis transmembrane regulator
Familial hypercholesterolemia	Low density lipoprotein (LDL) receptor
Emphysema	Alpha-1- antitrypsin
Thalassemia	Alpha or beta globin
Sickle-cell anemia	Beta-globin
Gaucher's disease	Glucocerebrosidase
Fanconi anemia	Complement group C gene delivery
Melanoma	Tumour necrosis factor (TNF)
Glioblastoma	Thymidine kinase
Duchenne muscular dystrophy	Dystrophin
Diabetes mellitus	Glucose transporter-2 (GLUT-2)

Table no. 1

Introduction of New Vectors

Various non-viral vectors that are presently being given consideration for gene therapy are naked DNA, oligonucleotides, lipoplexes and polyplexes dendrimers, etc. The advantages these vectors hold over viral vectors is low immunogenicity, rapid turnover and low toxicity. Most of these vectors are still in experimental stage and we are far from development of a perfect vector of gene therapy. Non-viral vectors can further be classified into those limited to in-vitro applications like calcium phosphate transfection which is the system of choice for transmitting plasmid DNA into variety of cell cultures. Another type of nonviral vectors are also there which have both in-vivo and in-vitro applications like cationic liposomes, etc¹².

Viral vectors for gene transfer: Viral vectors have been used in >70% of the clinical trials to date. Viruses are vehicles that efficiently transfer their therapeutic genes.

Adenoviral vectors: Adenoviral vectors are derived from Adenoviruses (ADV), DNA viruses with a linear double stranded genome (36 Kilo base pairs, Kbp), a non enveloped icosahedral capsid with characteristic morphology replicating in the nucleus and producing thousands of progeny virion released by cell lysis. The viral genome encodes about 50 viral proteins, 11 of which are structural and used to physically build the virion¹³.

Herpes virus vectors: Herpes virus vectors mainly derive from HSV type-1, a neurotoxic large DNA virus (152 Kbp, double stranded DNA) that comprises more than 80 genes categorized into essential and non-essential genes according to their requirement for viral replication. In its natural life cycle, HSV-1 is spread by contact, infects and replicates in skin membranes.

Retrovirus vectors: Retroviruses are a group of RNA containing viruses characterized by the employment of the unique molecular mechanisms which is an efficient transfer system. By the reverse transcriptase enzyme activity, the viral RNA genome to be transcribed into double stranded DNA that stable integration into host DNA¹⁴.

Non-viral vector for gene transfer:

Non-viral gene therapy is the introduction of therapeutic genes via plasmid DNA into target cells without the use of a virus. However, they demonstrate low transduction efficiencies, which make them less desirable. These techniques include microinjection, electroporation, nonoperation, gene gun, controlled, and chemical delivery methods.

Ormasil

The use of Ormasil (silica or modified organic silicate) is another non-viral method. The relative ease of working with silica has made it a good option for gene delivery. The most common method of using silica in gene therapy (due to its low toxicity) is the use of a combination of nanoparticles with amino silicones¹⁵.

Injection of Naked DNA

Injection of naked DNA is the simplest non-viral transfer method. Although clinical trials of this method have been successful, gene expression is much lower with it than with other methods. In addition to tests performed with plasmids, experiments have also been performed with naked PCR products.

Advantages of Gene Therapy

There are several advantages of gene therapy.

1. It offers hope.
2. Genetic disorders can be treated.
3. It may treat more than just disease.
4. It would create a new field of medicine.
5. Gene therapies aren't limited to humans.
6. Gene therapy is based on technology¹⁶.

Disadvantages of Gene Therapy

There are several disadvantages of gene therapy.

1. It is a costly treatment option.
2. Nature is adaptable.
3. It may unlock unethical forms of science.
4. Gene therapies have been stuck in trials for a generation for a good reason.
5. It may encourage gene doping¹⁷.

Physical Methods to Enhance Delivery

Gene Electroporation

The other terms used for electroporation are gene electro injection, gene electro transfer, electrically mediated gene therapy, electro gene transfer. Applying an electric field that is greater than the membrane capacitance will cause charges of opposite polarity to line up on either side of cell membrane thus forming a potential difference at a specific point on the cell surface. The pore of the membrane can be reversible based on the field strength and pulse duration. If it is reversible cells remain viable, otherwise cell death results as shown in following fig. no. 2

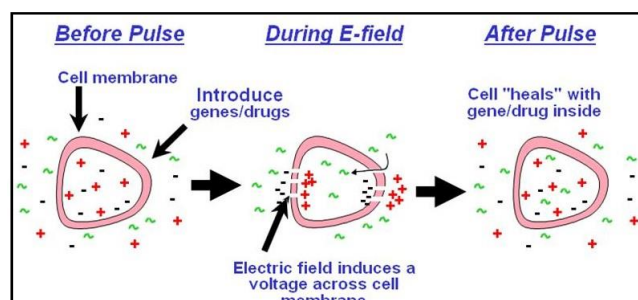


Fig no.2

Gene Gun

The use of particle bombardment, or the gene gun, is another physical method of DNA transfection. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells. Furthermore, researchers reported that if the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor¹⁸.

Sonoporation

Sonoporation is a method that uses ultrasonic frequencies to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells.

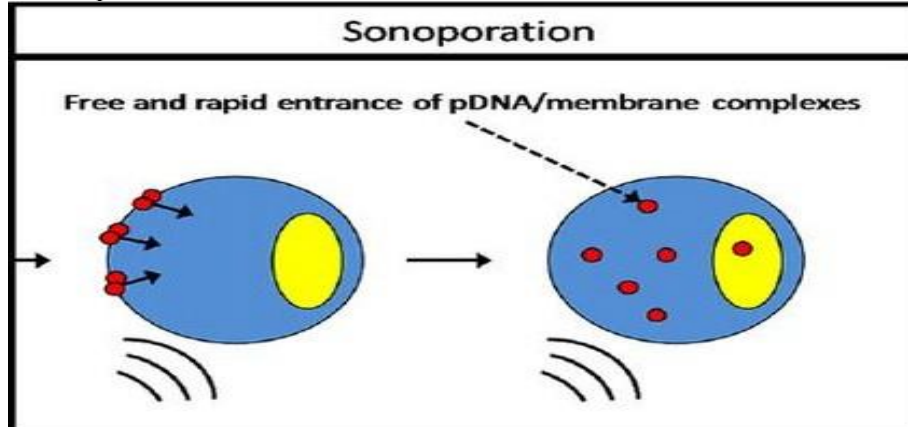
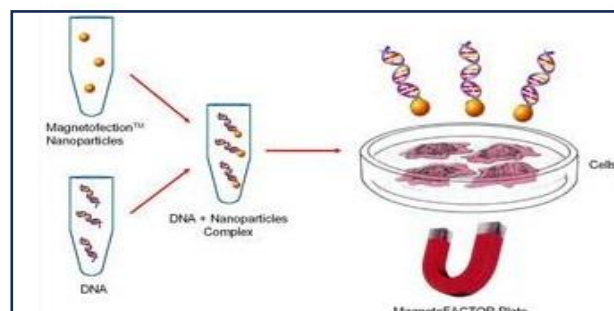


Fig. no. 3

Magnetofection

In a method termed magnetofection, the DNA is complexed to magnetic particles, and a magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer¹⁹.



Chemical Methods to Enhance Delivery of Gene

Oligonucleotides

The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Another uses small molecules of RNA called siRNA to signal the cell to cleave specific unique sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA and therefore expression of the gene. A further strategy uses double stranded oligodeoxynucleotides as a decoy for the transcription factors that are required to activate the transcription of the target gene. The transcription factors bind to the decoys instead of the promoter of the faulty gene, which reduces the transcription of the target gene, lowering expression²⁰.

Hybrid methods

The development of novel and safer vector tools for stable maintenance of therapeutic DNA and transgene products within a cell, especially in rapidly dividing cells (e.g., bone marrow derived cells), is of great interest to the research community. For instance therapies for these genetic diseases would benefit from such tools because they rely on stable production of the defective gene product and subsequent long-term phenotypic correction. Furthermore, these vectors would circumvent repeated vector administration necessary for life-long correction. Moreover, inconveniences for the patient due to repeated drug administration could be avoided. Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone. Other methods involve mixing other viral vectors with cationic lipids or hybridising viruses²¹.

Number of Tests Per Year

Number of Tests Per Year The number of tests carried out annually is significantly influenced by reports that indicate risks in this area; for example, in 2003 and 2007, the number of tests decreased, but in 2005, 2006, and 2008, considerably high numbers of clinical trials took place. There has also been a growing trend in the number of annual

trials since 2012. In recent years, an inadequate number of articles has been submitted to databases due to timeliness of publication, which has in turn led to a delay in obtaining information on most experiment²².

Fig no.5

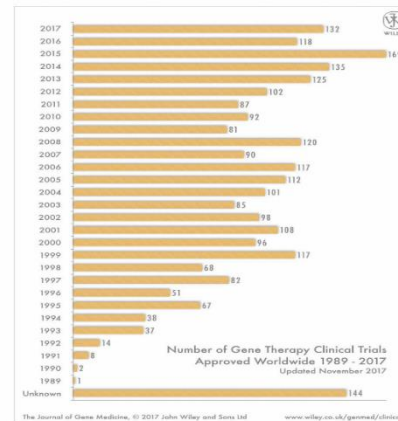
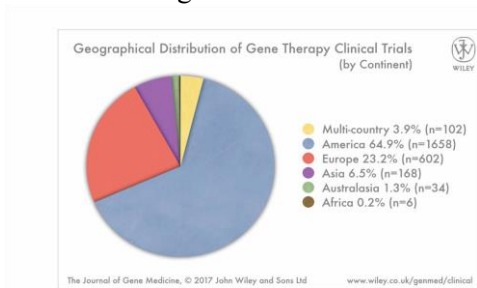


Fig no 6

Applications of Gene Therapy

Since 1989, the year of the first gene therapy clinical trial, over 1500 clinical studies have been conducted involving several tens of thousands of patients. If objectively evaluated, the overall clinical success of these trials has been modest so far. With some remarkable exceptions, most of the trials have encountered unanticipated technological and biological problems. In this evaluation, however, it should be taken into account that the vast majority of the diseases faced by gene therapy are life-threatening conditions, for which no conventional medical therapy exists, and that gene therapy is a completely new discipline, both conceptually and technically.

Indeed, 20 years after the first application, the possibility of success of gene therapy now appears much closer. This is a consequence of the significant improvements made in the development of both in vivo and ex vivo systems for gene delivery and the identification of novel classes of therapeutic genes. The recent results obtained by gene therapy of inherited blindness and of some neurodegenerative disorders, as well as the progress made in several other clinical trials, now encourage informed and firm optimism on the eventual success²³.

List of clinical conditions that submitted to gene therapy trials;

- In Parkinson's diseases (PD)
- In Alzheimer's disease (AD)
- In Diabetic Neuropathy
- In Metastatic Melanoma
- Cancer
- Atherosclerosis²⁴

Parkinson's Disease

According to independent reports, the effectiveness of gene therapy in Parkinson's disease (PD) has been proven. For example, in one of the proposed methods, the level of a chemical called GABA, the absence of which causes PD, is increased in the brain. In an experiment conducted on 45 volunteers with severe PD, tubes were placed in the areas of the brain associated with movement. Half of the participants were injected with viruses carrying the gene that increases GABA production, and the other half were given an innocuous saline solution (as the control group). After 6 months, those who underwent gene therapy showed a 23% improvement in movement ability, which was twice the improvement observed for those in the control group. The study discussed was a randomized controlled trial to investigate the improvement of advanced symptoms of PD using gene therapy. In the research, genes producing the chemical agent glutamic acid carboxylase (GAD) were transferred into the base ganglia cells, which are a set of cerebral areas controlling movement. The transferred GAD gene increased the level of a chemical messenger called GABA. The level of GABA in some parts of the basic ganglia is reduced in people with Parkinson's disease²⁵.

Alzheimer's Disease

Mental disorders are among the most common nervous system disorders, and Alzheimer's disease (AD) is the most common cause of dementia worldwide for which there are no effective therapies. AD and a number of frontotemporal dementias (FTDs) are collectively known as tauopathies, which are caused by the abundant accumulation of defective tau in the brain. Recent developments in gene therapy-based approaches, recombinant AAVs (rAAVs) in particular, have provided new tools for the study of AD and other neurological disorders.

In 2001, a clinical trial of nervous growth factor (NGF) gene therapy was conducted on Alzheimer's disease patients to determine whether decaying neuronal cells in AD, after the onset of the disease, still had the ability to respond to the neural growth factor. In the first attempt, a dysfunctioning nerve gene was transferred to an adult. Based on the results,

the decaying neuronal cells of the brain in AD responded to NGF. All patients showed a nutritional response to NGF; they grew axonal sprouts toward the source of NGF. The brains of 3 patients who underwent one-way gene transfer were examined in terms of the degree of treatability, and it was found that cholinergic neuronal hypertrophy occurred on the side treated with NGF ($P > 0.05$). In the cases of the 2 patients treated with AAV2-mediated and NGF gene transfer, functional markers and cellular signaling were activated²⁶.

Neurons with Tau damage (proteins that cause microtubule stability) as well as neurons without Tau damage expressed NGF, suggesting that decaying cells can be treated with gene therapy, resulting in the activation of the cellular signaling pathway. No NGF-related side effects were observed. NGF-induced sprouting continued for more than 10 years, longer than the expected assurance period²⁷.

Diabetic Neuropathy

In a study on a common disorder resulting from chronic diabetes, researchers found that gene therapy is promising in the treatment of diabetic polyneuropathy. Researchers in Boston found that intramuscular injection of a vascular endothelial growth factor (VEGF) gene may help diabetic neuropathy patients. This study included 39 patients who received 3 VEGF injections in one leg, plus 11 patients who received placebos. Legs and plantar pain, weakness, and balance problems are signs of diabetic neuropathy. A reduction in the tactile sense means that a foot ulcer may not be detected, and this can result in amputation. Most patients had relatively severe neuropathy and not much hope for recovery. "The VEGF gene used in this study was active and present without any packaging in the virus, which is a great and solid benefit," said Dr. Allan Ropper, Executive Director of the Department of the Neurology at Brigham and Boston Hospitals. The study showed that this form of gene transfer could be relatively safe, but before it can be introduced as a major treatment, further research is needed using a larger study population²⁸.

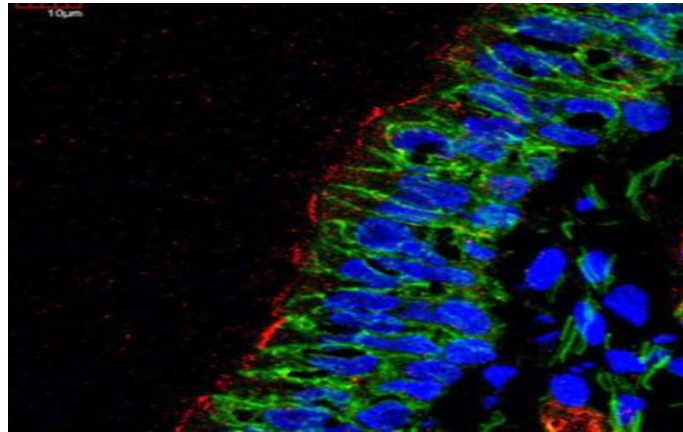


Fig no .7

Cancer Treatment

Progress in the genomics of humans over the past 2 decades has shown that cancer is caused by anomalies in the somatic cells of the host genome. These achievements have led many cancer researchers to use treatments based on genetic manipulation and modification to treat cancer and find a potential cure for the disease. Examples include gene therapy using viral (or bacterial) vectors or non-viral vectors, stimulating the immune system (immunomodulation) against tumor cells, manipulation of the tumor cell in order to reduce the tumor tissue, and increasing antigens for better detection of tumors by the immune system of the host. In general, the number of treatments with few side effects has been limited²⁹.

New generation viral or non-viral vectors significantly decrease risks associated with previous methods of cancer treatment, such as the integration of a retrovirus with a host genome with the risks of mutagenicity or malignancy, immune response against viruses, formation of tumors, drug resistance, or disease relapse. Several tumor-specific antibodies, genetically-modified immune cells, and vaccines have been developed and are currently available on the market; many others are being tested in clinical trials. Gene therapy is expected to play an important role as part of a multi-faceted treatment for cancer, together with other cancer treatments such as surgery, radiotherapy, and chemotherapy. The type and state of gene therapy are determined based on individual genome components, tumor characteristics, genetics, and host immune status in order to design a multifaceted treatment that is unique to the needs of each individual. Viral vectors are the main means of gene transfer in gene therapy for cancer. Particularly, the oncolytic viruses that selectively infect and kill cancer cells provide a more promising prospect. Technology for editing and modifying hereditary mutated genes, the interaction with stem cells for tissue regeneration, and the effective use of powerful immune responses to fight cancer also contribute to gene therapy revivification³⁰.

Future Prospectives

The field of gene therapy has received a lot of interest and shows great promise since the first clinical experiment was carried out. There have been significant public and investments from the commercial sector in addition to rising levels

of research effort. Proofs of concept for several possible clinical applications have been produced via a multitude of preclinical animal model studies³¹. Significant progress has also been achieved in enhancing vector production and design as well as in comprehending vector biology. A novel treatment for Huntington's disease might involve RNA interference or gene silencing. Cells employ tiny segments of double stranded RNA, known as short, interfering RNAs or si RNAs, to break down RNA belonging to a certain sequence. An aberrant protein product will result if a si RNA is made to match the RNA that was copied from a defective gene. New gene therapy approach repairs errors in messenger RNA derived from defective genes. Technique has potential to treat the blood disorder thalassemia, cystic fibrosis, and some cancers³².

Conclusion

Gene therapy is thought to be the long-term cure for hereditary illnesses. However, due to a number of inherent complications, gene therapy is not as straightforward as it first seems. Depending on how it is used, gene therapy can be both advantageous and detrimental. Gene therapy offers the benefit of curing those who are born with a genetic disease or who develops fatal illnesses like as cancer, AIDS, etc. To promote gene therapy applications, the public sector, the government, and the scientific community should work together. A breakthrough could occur at any time, and one day gene therapy could be used as one of the therapeutic options for nearly all diseases.

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

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