International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Review Article Open Access

Gene Therapy: A Diverse Biomedical Techniques

K. Lavanya*, Ramesh Dhani, T. Sairajitha, K. Sravani, G. Tharunasree, G. Gnanaprasuna

Ratnam Institute of Pharmacy, Pidathapolur, Muthukur (M), Nellore, Andhra Pradesh, India-524346

A B S T R A C T

In recent years there have been a number of technological break throughs that have allowed for clinical trails in gene therapy to be initiated. In combination with the genome invetiatin, the potential for new therapeutics is limitless. Alltough an uncommon enormous among of information has been obtained in a relatively short period of time. Gene therapy is not get ready for wide scale practice. Some of the success and obstascles that remain are summerised in this report.

Keywords: Gene therapy, genetic manipulation, DNA, Haemophilia, Chromosomes.

A R T I C L E I N F O

CONTENTS

Article History: Received 27 November 2014, **Accepted** 29 January 2015, **Published Online** 15 March 2015

***Corresponding Author**
K. Lavanya
Ratnam Institute of Pharmacy,
Pidathapolur, Muthukur (M), Nellore,
Andhra Pradesh, India-524346
Manuscript ID: IJCTPR2446

PAPER-QR CODE

Citation: K. Lavanya, et al. Gene Therapy: A Diverse Biomedical Techniques. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(2): 855-862.

1. Introduction

Gene therapy is the process of inserting genes into cells to treat diseases. the newly introduced genes will encode proteins and correct the genetic diseases that occur in genetic diseases. The gene therapy primarily involves genetic manipulation in animals or humans to correct a disease, and keep the organism in good health. The initial experiments in gene therapy are carried out in animals and then in humans. The goal of researchers is to benefit the mankind and improve their health [3,4]

The basis of gene therapy:

Proteins have a lot of important functions in our body. For example, they function as building blocks. They transfer messages and break down waste materials. The blueprints of proteins are written in our genetic material, the DNA. We call these blue prints 'genes'. When something's wrong

with a blue print, or gene, properly functioning proteins cannot be produced. They work less, or not at all. This can have far reaching effects. Illnesses like Haemophilia (an hereditary disorder of blood clotting) and cystic fibrosis are examples. The idea with gene therapy is that when genes contain certain faults, we should be able to do something about it. When we replace or repair the gene, this should make things right.

One Gene, One Protein: The Basics of Gene Therapy

Understanding this medical treatment requires a working knowledge of genes. A gene refers to a single unit of hereditary information a factor that controls some specific activity or trait. A gene is a section of DNA that codes for a defined biochemical function, usually production of a protein.It consists of the coded information of the DNA in the sequences of the bases. They are the pieces of information coded in DNA on their own. These genes are responsible for the characteristics and behavior of a particular individual. Genes exist on chromosomes**,** which themselves reside in the nuclei of our cells. Chromosomes contain long chains of DNA built with repeating subunits known as nucleotides**.** That means a single gene is a finite stretch of DNA with a specific sequence of nucleotides. Those nucleotides act as a blueprint for a specific protein, which gets assembled in a cell using a multistep process. The first step, known as transcription, begins when a DNA molecule unzips and serves as a template to create a single strand of complementary messenger RNA. The messenger RNA then travels out of the nucleus and into the cytoplasm, where it attaches to a structure called the ribosome. There, the genetic code stored in the messenger RNA, which itself reflects the code in the DNA, determines a precise sequence of amino acids. This step is known as translation**,** and it results in a long chain of amino acids -- a protein. Proteins are the workhorses of cells. They help build the physical infrastructure, but they also control and regulate important metabolic pathways. If a gene malfunctions -- if, say, its sequence of nucleotides gets scrambled -- then its corresponding protein won't be made or won't be made correctly. Biologists call this **a**mutation**,** and mutations can lead to all sorts of problems, such as cancer and phenylketonuria. Gene therapy tries to restore or replace a defective gene, bringing back a cell's ability to make a missing protein. On paper, it's straightforward: You simply insert the correct version of a gene into a strand of DNA. In reality, it's a little more complicated because cells require some outside assistance in the form of a virus. You probably think of viruses as agents that cause infections -- smallpox, influenza, rabies or AIDS. In gene therapy, scientists use these tiny living-but-not-living particles to give a cell a genetic makeover. In the next section, we'll explore which viruses are used and why.

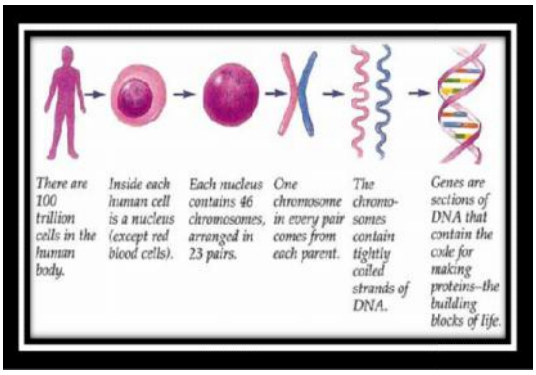

Figure 1

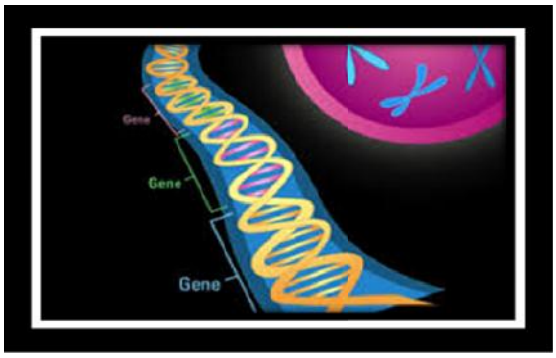

Figure 2

Functions of a gene:

Most genes contain the information needed to make functional molecules called proteins. (A few genes produce other molecules that help the cell assemble proteins.) The journey from gene to protein is complex and tightly controlled within each cell. It consists of two major steps: transcription and translation. Together, transcription and translation are known as gene expression. During the process of transcription, the information stored in a gene's DNA is transferred to a similar molecule called RNA (ribonucleic acid) in the cell nucleus. Both RNA and DNA are made up of a chain of nucleotide bases, but they have slightly different chemical properties. The type of RNA that contains the information for making a protein is called messenger RNA (mRNA) because it carries the information, or message, from the DNA out of the nucleus into the cytoplasm. Translation, the second step in getting from a gene to a protein, takes place in the cytoplasm. The mRNA interacts with a specialized complex called a ribosome, which "reads" the sequence of mRNA bases. Each sequence of three bases, called a codon, usually codes for one particular amino acid. (Amino acids are the building blocks of proteins.) A type of RNA called transfer RNA (tRNA) assembles the protein, one amino acid at a time. Protein assembly continues until the ribosome encounters a "stop" codon (a sequence of three bases that does not code for an amino acid). The flow of information from DNA to RNA to proteins is one of the fundamental principles of molecular biology. It is so important that it is sometimes called the "central dogma."

Genome: A genome is an organism's complete set of DNA, including all of its genes. Each genome contains all of the information needed to build and maintain that organism. In humans, a copy of the entire genome—more than 3 billion DNA base pairs is contained in all cells that have a nucleus. Through the processes of transcription and translation, information from genes is used to make proteins.

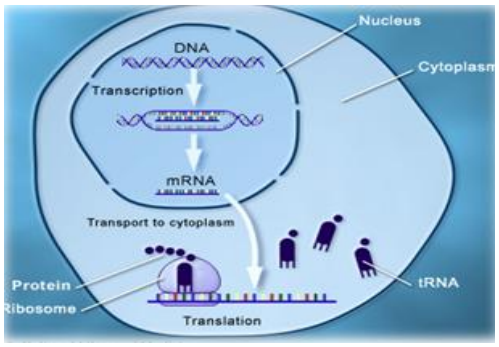

Figure 3

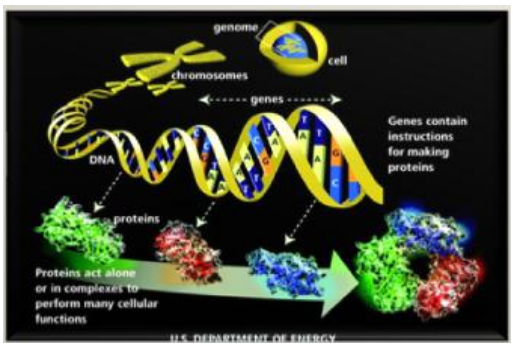

Figure 4

Genes and Their Mutations Leading To Diseases:

A gene mutation is a permanent change in the DNA sequence that makes up a gene. Mutations range in size from a single DNA building block (DNA base) to a large segment of a chromosome.

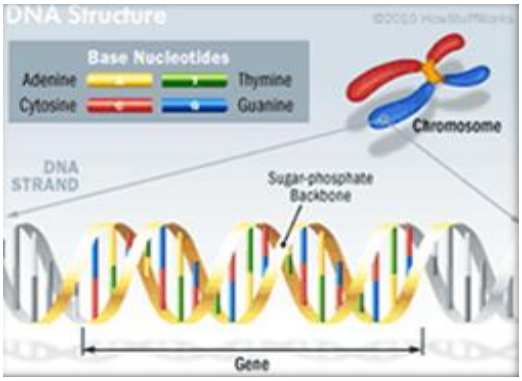

Figure 5

Gene mutations occur in two ways: they can be inherited from a parent or acquired during a person's lifetime. Mutations that are passed from parent to child are called hereditary mutations or germline mutations (because they are present in the egg and sperm cells, which are also called germ cells). This type of mutation is present throughout a person's life in virtually every cell in the body. Mutations that occur only in an egg or sperm cell, or those that occur just after fertilization, are called new (de novo) mutations. De novo mutations may explain genetic disorders in which an affected child has a mutation in every cell, but has no family history of the disorder. Acquired (or somatic) mutations occur in the DNA of individual cells at some time during a person's life. These changes can be caused by environmental factors such as ultraviolet radiation from the sun, or can occur if a mistake is made as DNA copies itself during cell division. Acquired mutations in somatic cells (cells other than sperm and egg cells) cannot be passed on to the next generation. Mutations may also occur in a single cell within an early embryo. As all the cells divide during growth and development, the individual will have some cells with the mutation and some cells without the genetic change. This situation is called mosaicism. To function correctly, each cell depends on thousands of proteins to do their jobs in the right places at the right times,gene mutations prevent one or more of these proteins from working properly. By changing a gene's instructions for making a protein, a mutation can cause the protein to malfunction or to be missing entirely· **[5,6,7,8,9]**

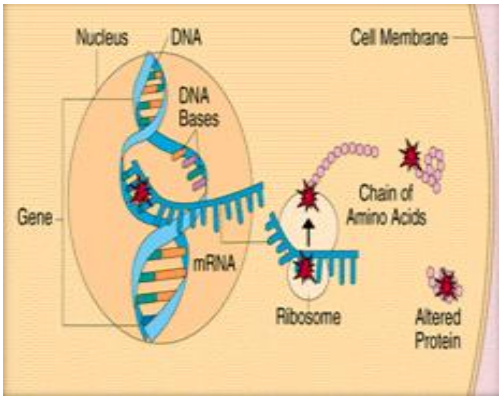

Figure 6

2. Technique Involved in Gene Therapy

Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein. If a mutated gene causes a necessary protein to be faulty or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein.

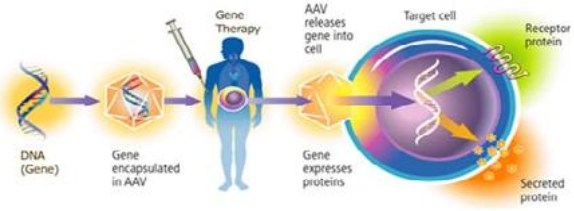

Figure 7

Vectors in gene therapy

A gene that is inserted directly into a cell usually does not function. Instead, a carrier called **a** vector is genetically engineered to deliver the gene. Gene therapy utilizes the delivery of DNA into cells, which can be accomplished by

several methods, summarized below. The two major classes of methods are those that use recombinant viruses (sometimes called biological nanoparticles or viral vectors) and those that use naked DNA or DNA complexes (non-viral methods).To be successful, a vector must:

a. Target the right cells. If you want to deliver a gene into cells of the liver, it shouldn't wind up in the big toe.
b. Integrate the gene in the cells. You need to ensure that the gene integrates into, or becomes part of, the host cell's genetic material, or that the gene finds another way to survive in the nucleus without being trashed.
c. Activate the gene. A gene must go to the cell's nucleus and be "turned on," meaning that it is transcribed and translated to make the protein product it encodes. For gene delivery to be successful, the protein must function properly.
d. Avoid harmful side effects. Any time you put an unfamiliar biological substance into the body, there is a risk that it will be toxic or that the body will mount an immune response against it.

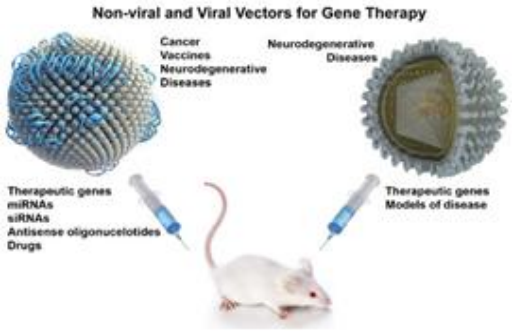

Figure 8

Viral vectors of gene therapy:

All viruses bind to their hosts and introduce their genetic material into the host cell as part of their replication cycle. This genetic material contains basic 'instructions' of how to produce more copies of these viruses, hacking the body's normal production machinery to serve the needs of the virus. The host cell will carry out these instructions and produce additional copies of the virus, leading to more and more cells becoming infected. Some types of viruses insert their genome into the host's cytoplasm, but do not actually enter the cell. Others penetrate the cell membrane disguised as protein molecules and enter the cell.

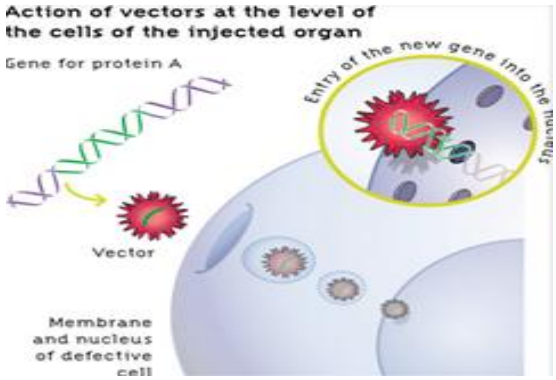

Figure 9

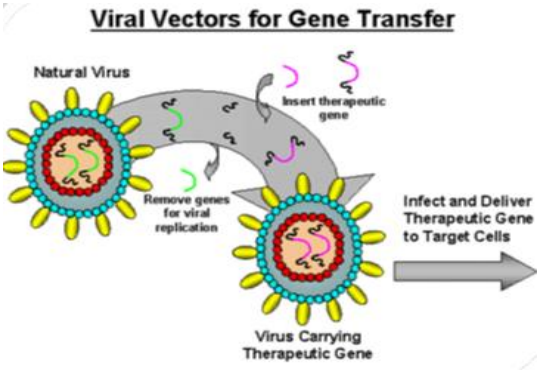

Figure 10

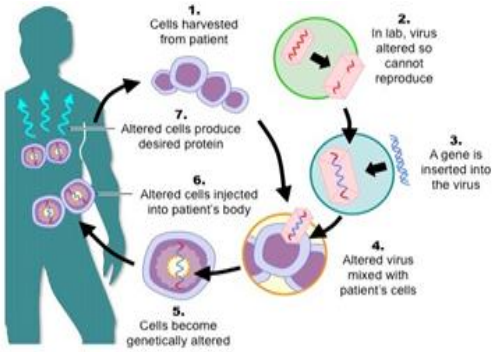

Figure 11

Non Viral Vectors of Gene Therapy:

Viruses may effectively deliver genetic material into a patient's cells, but they have some limitations. Some of these limitations can be overcome by using non-viral vectors. One type of non-viral vector is a circular DNA molecule called a plasmid. In nature, bacteria use plasmids to transfer share genes with one another. To make it easier for them to enter cells, gene-therapy plasmids are sometimes packaged inside of "liposomes," small membrane-wrapped packets that deliver their contents by fusing with cell membranes. The disadvantage of plasmids and liposomes is that they are much less efficient than viruses at getting genes into cells. The advantages are that they can carry larger genes, and most don't trigger an immune response. Synthetic vectors called virosomes are essentially liposomes covered with viral surface proteins. They combine the carrying capacity and immune advantages of plasmids with the efficiency and specificity of viruses. The viral proteins interact with proteins on the target-cell surface, helping the virosome fuse with the cell membrane and dump its contents into the cell. Different types of viral proteins can target specific types of cells. [14,15]

Ethics and Safety Regarding Gene Therapy:

Safety regarding Gene Therapy:

Gene therapy is under study to determine whether it could be used to treat disease. Current research is evaluating the safety of gene therapy; future studies will test whether it is an effective treatment option. Several studies have already shown that this approach can have very serious health risks, such as toxicity, inflammation, and cancer. Because the techniques are relatively new, some of the risks may be unpredictable; however, medical researchers, institutions, and regulatory agencies are working tso ensure that gene therapy research is as safe as possible.

Ethical issues surrounding gene therapy:

Current gene therapy research has focused on treating individuals by targeting the therapy to body cells such as bone marrow or blood cells. This type of gene therapy cannot be passed on to a person's children. Gene therapy could be targeted to egg and sperm cells (germ cells), however, which would allow the inserted gene to be passed on to future generations. This approach is known as germline gene therapy. The idea of germline gene therapy is controversial. While it could spare future generations in a family from having a particular genetic disorder, it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known. Because people who would be affected by germline gene therapy are not yet born, they can't choose whether to have the treatment. Because of these ethical concerns, the U.S. Government does not allow federal funds to be used for research on germline gene therapy in people. [10]

History

Gene therapies had been administered to more than 4000 patients by the start of 2001; however these all were phase1 trials testing to see if the approach was safe or atleast less dangerous than the disease they were suffering from. In the early 1970s, scientists proposed "gene surgery" for treating inherited diseases caused by faulty genes. The idea was to take out the disease-causing gene and surgically implant a gene that functioned properly. Although sound in theory, scientists, then and now, lack the biological knowledge or technical expertise needed to perform such a precise surgery in the human body. However, in 1983, a group of scientists from Baylor College of Medicine in Houston, Texas, proposed that gene therapy could one day be a viable approach for treating Lesch-Nyhan disease, a rare neurological disorder.

The scientists conducted experiments in which an enzyme-producing gene (a specific type of protein) for correcting the disease was injected into a group of cells for replication. The scientists theorized the cells could then be injected into people with Lesch-Nyhan disease, thus correcting the genetic defect that caused the disease. Further studies of DNA and chromosomes (where genes reside) showed that specific genetic abnormalities in one or more genes occurred in successive generations of certain family members who suffered from diseases like intestinal cancer, bipolar disorder, Alzheimer's disease**,** heart disease, diabetes, and many more. Currently, there are a host of new gene therapy agents in clinical trials. Presently, gene therapies for the following diseases are being developed: cystic fibrosis (using adenoviral vector), HIV infection (cell-based), malignant melanoma (cell-based), Duchenne muscular dystrophy (cell-based), hemophilia B (cell-based), kidney cancer (cell-based), Gaucher's Disease (retroviral vector), breast cancer (retroviral vector), and lung cancer (retroviral vector). When a cell or individual is treated using gene therapy and successful incorporation of engineered genes has occurred, the cell or individual is said to be *transgenic*. [12, 13]

3. Types of gene therapy

It is basically done in two ways

***Ex- vivo*:**

Well developed method involves identifying suitable target cells in an affected patient, removing them carrying the gene transfer in-vitro and returning the genetically and phenotypically corrected cells into the patient.

In- vivo:

Involves introduction of a vector directly into the patient, that is targeted to the appropriate tissue or organ into the patient's body. It is simpler, and more easily delivered of the two potential approaches of the gene therapy.

***Ex-vivo* Vs *In-vivo*:**

Genes can be delivered into a group of cells in a patient's body in two ways. The first, called *in vivo* (in VEE-voh), is to inject the vector directly into the patient, aiming to target the affected cells. The second, called *ex vivo* (ex VEE-voh), is to deliver the gene to cells that have been removed from the body and are growing in culture. After the gene is delivered, integration and activation are confirmed, and the cells are put back into the patient. *Ex-vivo* approaches are less likely to trigger an immune response, because no viruses are put into patients. They also allow researchers to make sure the cells are functioning properly before they're put in the patient. Several gene therapy successes use *ex vivo* gene delivery as an alternative to bone marrow transplants.

Based on the type of cells which are used for gene therapy, it is of two types

Germ line gene therapy

This is changing the germ cells, which make sperm/ova. It shows permanent effect on the individuals which are the descendents of whoever had the therapy. In this a fertilized egg is provided with a correct version of the relevant gene and re implantation into mother, therefore the change is heritable and can be passed on to later generations. This is used in animals and trials have been done in animals like mouse and chimpanzees. This technique of gene therapy done in animals is called Transgenic technology. This technology may lead to generation of new mutant species. Trials on humans have been considered unethical and has not legally been tried.

Somatic cell therapy

In this the gene is introduced only in somatic cells especially of those tissues in which the expression of concerned gene is critical for health. Expression of

concerned gene eliminates symptoms of the disorder. But this effect is not heritable, as it doesn't involves the germ line. It alters all the other non-germ cells of the body (somatic cells).changing them doesn't affect germ cells, but affects the engineered person. Currently somatic cell therapy is only option for humans, and clinical trials have started mostly for the treatment of cancer and blood disorders. Somatic germ therapy can be aimed to correct a genetic or non -genetic defects. Current therapeutic targets include both the categories· [15]

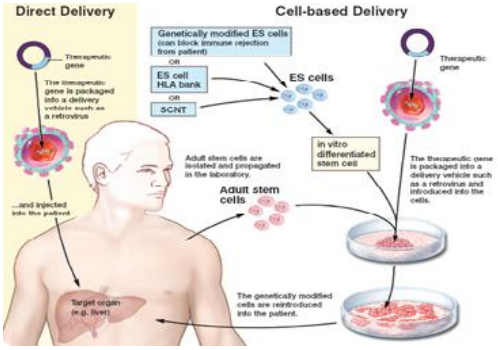

Figure 12

Strategies: The first, in which, DNA is inserted into the genome to replace a missing gene product. The later in which an antisense gene inhibits the expression of the dominant gene.

Gene Augmentation therapy:

In this DNA is added to the genome to replace a lost/ missing function and can be derived as gene augmentation therapy. Transferred genes may be suitably integrated into the genome, where there is potential to permanently correct the defect especially if stem cells are transformedor may be maintained episomally (in which case there is an inevitable decay in the may need to be repeated). Several GAT trials are underway including Cystic fibrosis, Adenosine deaminased eficiency (ADA) and Familial hyper cholesterolaemia.

Targeted gene transfer:

TGT uses homologuous recombination to replace the endogenous genes with functionally introduced gene, the first case of such gene transfer was used to disrupt the human β2 globin gene in cultured cells. However targeted correction (removing mutant/abnormal allele)is a very inefficient process in practice, even in cultured cells and its application to the correction of genetic defects in somatic cells especially in vitro awaits further technical improvements.

Gene inhibition Therapy:

Approaches for gene inhibiting the expression of specific genes have become an important therapeutic parameter of gene based therapy of many acquired diseases. Antisense oligonucleotides and certain classes of ribosomes are in use or at various stages of development to treat a wide range of disease process including viral infections and cancer. Fomivirsen, a specific anti-sense oligonucleotide designed for the treatment of ocular cyto-megalovirus infection was the first nucleic acid drug to receive FDA approval for clinical use.

Ectopic synthesis of therapeutic protein: Deficiency of a variety of growth factors and peptide hormones are potentially amenable to treatment using the paradigm of ectopic gene expression .This approach involves delivery of a gene to evoke expression of a circulatory protein from a tissue that normally does not synthetise the product. This strategy has been used to induce expression of coagulation factors (8 &9), growth factors (IGF-1), erythropoietin and peptide hormones. Main issue with SCT is getting DNA into all cells that you want to treat. [16, 17]

Methods of Treatment of diseases by Gene Therapy:

A few techniques are aimed at replace a defective copy of a gene with a working copy. The term SMaRT™ stands for "Spliceosome-Mediated RNA Trans-splicing." This technique targets and repairs the messenger RNA (mRNA) transcripts copied from the mutated gene. Rather than attempting to replace the entire gene, this technique repairs just the section of the mRNA transcript that contains the mutation. Several different viral vectors have been developed to repair mutations directly in the DNA. This gene editing technique uses enzymes designed to target specific DNA sequences. The enzymes cut out the faulty sequence and replace it with a functional copy.

Gene silencing: It is an approach used to turn a gene off so that no protein is made from it. Gene-silencing approaches to gene therapy can target a gene's DNA directly, or they can target mRNA transcripts made from the gene. Gene editing, in addition to repairing mutations as described above, can be used to introduce a mutation into a gene's DNA sequence so that no protein is made from it.

Diseases targeted by gene therapy:

The vast majority (82.7%) of gene therapy clinical trials to date have addressed cancer, cardiovascular disease and inherited monogenic diseases; the first two because of their enormous prevalence, impact and potentially fatal outcomes, the latter has an obvious appeal and rationale**.**

1. Cancer
2. Cardiovascular diseases
3. Inherited monogenic diseases

Gene Therapy Successes:

Below are some gene therapy success stories. Successes represent a variety of approaches different vectors, different target cell populations, and both in vivo and ex vivo approaches to treating a variety of disorders.

Immune Diseases:

Severe Combined Immune Deficiency (SCID) was one of the first genetic disorders to be treated successfully with gene therapy, proving that the approach could work. However, the first clinical trials ended when the viral vector triggered leukemia (a type of blood cancer) in some patients. Since then, researchers have begun trials with new, safer viral vectors that are much less likely to cause cancer. Adenosine deaminase (ADA) deficiency is another inherited immune disorder that has been successfully treated with gene therapy. In multiple small trials, patients' blood stem cells were removed, treated with a retroviral vector to deliver a functional copy of the ADA gene, and then returned to the patients. For the majority of patients in these trials, immune function improved to the point that

they no longer needed injections of ADA enzyme. Importantly, none of them developed leukemia.

Hereditory Blindness:

Gene therapies are being developed to treat several different types of inherited blindness—especially degenerative forms, where patients gradually lose the light-sensing cells in their eyes. Encouraging results from animal models (especially mouse, rat, and dog) show that gene therapy has the potential to slow or even reverse vision loss. The eye turns out to be a convenient compartment for gene therapy. The retina, on the inside of the eye, is both easy to access and partially protected from the immune system. And viruses can't move from the eye to other places in the body. Most gene-therapy vectors used in the eye are based on AAV (adeno-associated virus). In one small trial of patients with a form of degenerative blindness called LCA (Leber congenital amaurosis), gene therapy greatly improved vision for at least a few years. However, the treatment did not stop the retina from continuing to degenerate. In another trial, 6 out of 9 patients with the degenerative disease choroideremia had improved vision after a virus was used to deliver a functional REP1 gene.

Hemophilia:

People with hemophilia are missing proteins that help their blood form clots. Those with the most-severe forms of the disease can lose large amounts of blood through internal bleeding or even a minor cut. In a small trial, researchers successfully used an adeno-associated viral vector to deliver a gene for Factor IX, the missing clotting protein, to liver cells. After treatment, most of the patients made at least some Factor IX, and they had fewer bleeding incidents.

Blood Diseases:

Patients with beta-Thalassemia have a defect in the beta-globin gene, which codes for an oxygen-carrying protein in red blood cells. Because of the defective gene, patients don't have enough red blood cells to carry oxygen to all the body's tissues. Many who have this disorder depend on blood transfusions for survival.

Fat metabolism Disorder:

In 2012, Glybera became the first viral gene-therapy treatment to be approved in Europe. The treatment uses an adeno-associated virus to deliver a working copy of the LPL (lipoprotein lipase) gene to muscle cells. The LPL gene codes for a protein that helps break down fats in the blood, preventing fat concentrations from rising to toxic levels.

Cancer

Gene therapy holds great promise for the treatment of cancer. Several promising gene-therapy treatments are under development for cancer. One, a modified version of the herpes simplex 1 virus (which normally causes cold sores) has been shown to be effective against melanoma (a skin cancer) that has spread throughout the body. The treatment, called T-VEC, uses a virus that has been modified so that it will (1) not cause cold sores; (2) kill only cancer cells, not healthy ones; and (3) make signals that attract the patient's own immune cells, helping them learn to recognize and fight cancer cells throughout the body. The virus is injected directly into the patient's tumors. It replicates (makes more of itself) inside the cancer cells until they burst, releasing more viruses that can infect additional cancer cells.

Parkinson's Disease:

Patients with Parkinson's disease gradually lose cells in the brain that produce the signaling molecule dopamine. As the disease advances, patients lose the ability to control their movements. A small group of patients with advanced Parkinson's disease were treated with a retroviral vector to introduce three genes into cells in a small area of the brain. These genes gave cells that don't normally make dopamine the ability to do so. After treatment, all of the patients in the trial had improved muscle control. [18]

4. Conclusion

I hope this assignment has provided you with an insight on gene therapy and its advantages and disadvantages. Gene therapy is an extremely complex topic that involves not only science problems, but many ethical concerns as well. As scientists proceed with their medical advancements, they must understand the ethical and moral implications of their advancements.

5. References

1. Sheridan C."Gene therapy finds its niche". *Nature Biotechnology*, **2011**, 29 (2):121–128. Doi:10. 1038 /nbt.1769.
2. Maguire AM, Simonelli F, Pierce EA, Pugh EN, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J. "Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis". *New England Journal of Medicine*, **2008**, 358(21): 2240–2248. Doi:10.1056/NEJMoa 0802315.
3. Simonelli F, Maguire AM, Testa F, Pierce EA, Mingozzi F, Bennicelli JL, Rossi S, Marshall K, Banfi S, Surace EM, Sun J, Redmond TM, Zhu X, Shindler KS, Ying GS, Ziviello C, Acerra C, Wright JF, McDonnell JW, High KA, Bennett J, Auricchio A."Gene Therapy for Leber's Congenital Amaurosis is safe and Effective through 1.5 Years after Vector Administration. *Molecular Therapy*, **2009**, 18(3): 643–650. Doi:10.1038/mt.2009.277.
4. Cideciyan AV, Hauswirth WW, Aleman TS, Kaushal S, Schwartz SB, Boye SL, Windsor EA, Conlon TJ, Sumaroka A, Roman AJ, Byrne BJ, Jacobson SG. Vision 1 Year after Gene Therapy for Leber's Congenital Amaurosis. *New England*

Journal of Medicine, **2009,** 361 (7): 725–727. Doi:10.1056/NEJMc0903652**.**

5. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, Petersen-Jones S, Bhattacharya SS, Thrasher AJ, Fitzke FW, Carter BJ, Rubin GS, Moore AT, Ali RR. Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis. *New England Journal of Medicine*, **2008**, 358 (21): 2231–2239. Doi:10.1056/NEJMoa 0802268.
6. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. 20 years of gene therapy for SCID. *Nature Immunology*, **2010**, 11 (6): 457–460. Doi:10.1038 /ni0610-457.
7. Ferrua F, Brigida I, Aiuti A. Update on gene therapy for adenosine deaminase-deficient severe combined immunodeficiency. *Current Opinion in Allergy and Clinical Immunology*, **2010,** 10(6): 551–556.
8. Cartier N, Aubourg P. "Hematopoietic Stem Cell Transplantation and Hematopoietic Stem Cell Gene Therapy in X-Linked Adreno-leukodystrophy. *Brain Pathology*, **2009**, 20(4): 857–862.
9. LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, Kostyk SK, Thomas K, Sarkar A, Siddiqui MS, Tatter SB, Schwalb JM, Poston KL, Henderson JM, Kurlan RM, Richard IH, Van Meter L, Sapan CV, During MJ, Kaplitt MG, Feigin A. AAV2-GAD gene therapy for advanced Parkinson's disease: A double-blind, sham-surgery controlled, randomised trial. *The Lancet Neurology*, **2011**, 10(4): 309–319.
10. Gene therapy deserves a fresh chance. *Nature*, **2009**, 461(7268): 1173–2009. Doi:10.1038/4611 173a.
11. Pezzoli D, Chiesa R, De Nardo L, Candiani G. We still have a long way to go to effectively deliver genes. *J. Appl Biomater Funct Mater*, **2012**, 10(2): 82–91. Doi:10.5301/JABFM.2012.9707.
12. Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M. Viral vectors: a look back and ahead on gene transfer technology. *New Microbiol.* **2013**, 36 (1): 1–22.PMID 23435812.
13. Gothelf A, Gehl J. What you always needed to know about electroporation based DNA vaccines**.** *Hum Vaccin Immunother*, **2012**, 8 (11): 1694–702. Doi:10.4161/hv.22062. PMC 3601144.
14. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. *Nature Reviews Genetics*, **2010**, 11(9): 636–646.Doi:10.1038/nrg2842.
15. Mavilio F, Ferrari G. Genetic modification of somatic stem cells. The progress, problems and prospects of a new therapeutic technology. *EMBO Rep*. **2008**, 9(1): S64–9. Doi:10.1038/embor. 2008. 81.
16. Strachnan, T. and Read, A. P. (**2004**) *Human Molecular Genetics*, 3rd Edition, Garland Publishing, pp. 616, ISBN 0815341849.
17. Woods NB, Bottero V, Schmidt M, von Kalle C, Verma IM. Gene therapy: Therapeutic gene causing lymphoma. *Nature*, **2006**, 440(7088): 1123.Doi:10.1038/4401123a. PMID 16641981.
18. Thrasher AJ, Gaspar HB, Baum C, Modlich U, Schambach A, Candotti F, Otsu M, Sorrentino B, Scobie L, Cameron E, Blyth K, Neil J, Abina SH, Cavazzana-Calvo M, Fischer A. Gene therapy: X-SCID transgene leukaemogenicity. *Nature*, **2006**, 443(7109): E5–E6; discussion E6–7. Doi:10.1038/ nature05219.