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A Review on Gene Therapy

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Abstract

In general, the idea of gene therapy is the introduction of genetic material into a cell, tissue, or entire organ with the intention of treating a disease or, at the very least, enhancing the patient's clinical condition .The creation of delivery mechanisms capable of efficiently transferring genes across a range of tissues without inducing any related harmful effects is a critical component in the success of gene therapy .At the moment, vectors based on a variety of viral systems—such as retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses—represent the best option for effective gene delivery .Clinical trials to treat genetic disorders and acquired diseases are based on the favorable results of their evaluation in animal models about their toxicity and performance .Even though these trials have shown some initial success, vector development is still a major challenge for enhanced gene therapy methods (2).

Key words: adenovirus, AAV, retrovirus/lentivirus, viral vectors, and clinics.

Introduction

Since the early 1980s, advances in molecular biology have been made. It has been established that human genes can be sequenced and cloned. Researchers are looking for new ways to produce proteins in diabetic patients more easily. Modified bacteria can be injected into patients who are unable to produce them naturally. They are concentrating on diseases caused by single-gene defects, such as sickle-cell anemia, hemophilia, cystic fibrosis, and muscular dystrophy.(1)

Gene Therapy History and Future

Hemophilia and gene therapy In pre-clinical and early clinical gene therapy, band other inherited plasma protein deficits have demonstrated considerable promise for the future. The girl received treatment at the National Institutes of Health's Clinical Center in Bethesda, Maryland, on September 14, 1990.

The procedures were completed by Dr. W. French Anderson and his colleagues at the health center.

White blood cells were taken out of the body. Following the implantation of genes that generate ADA, the cells were returned to the girl's body .There was a noticeable improvement in the girl's immune system .Meanwhile, the triple therapy for a number of illnesses is still ongoing .Patients with melanoma and skin cancer used gene therapy as treatment.(6)

Principles of genetic therapy:

A defective gene can be converted to a normal gene by homologous recombination; an abnormal gene can be matched with a normal gene by selected reverse mutation, which restores the gene to its normal function; the degree to which a gene is switched on or off can

change clinical. Through homologous recombination, a normal gene could be substituted with an aberrant one.(6)

One potential repair method for the aberrant gene is selected reversal mutation, which restores the gene to its typical function.

One possible change to a gene's regulation could be how much of it is turned on or off. (6)

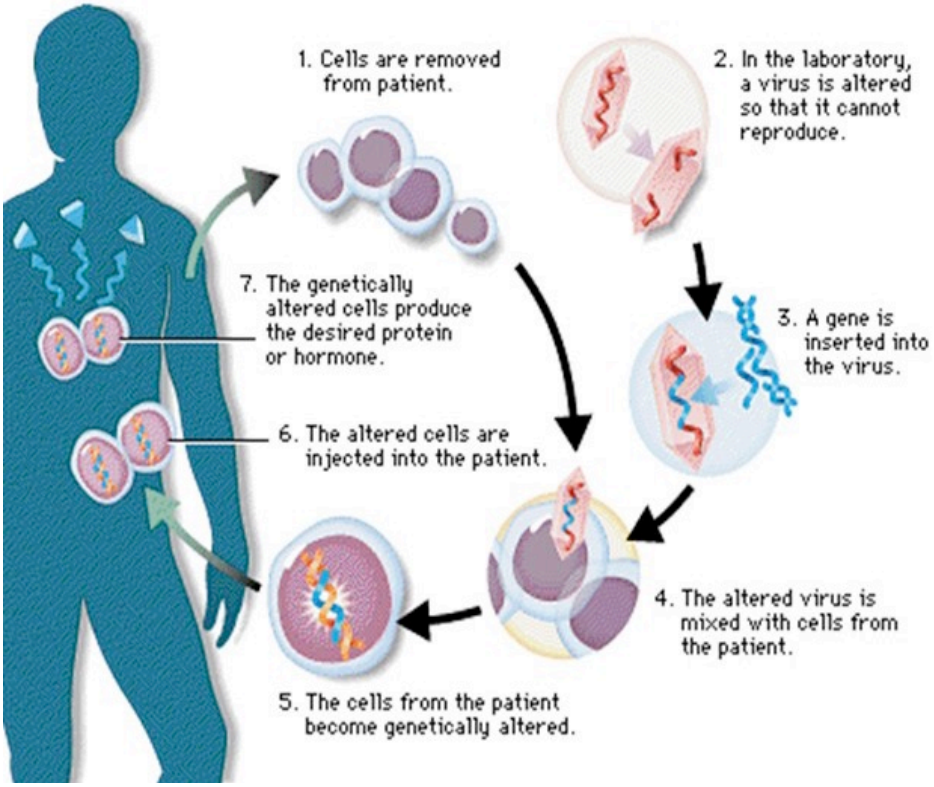

Figure 1: Flow chart shows gene therapy.

Approaches:

Two approaches have been proposed to transfer genes into a cell, according tol. (i) non-viral vectors and (ii) viral vectors . Currently, the viral vectors have been used in 70% of the clinical trials. Even though viral vectors are incredibly effective in spreading genes, they can also pose some threats to public health. Transduction is the process by which genes are transferred and is facilitated by viral vectors. Adenoviruses (e.g., HIV), lentiviruses, kerpes simplex viruses, retroviruses (e.g., HIV), and hybrid viruses (which combine the advantageous traits of multiple viruses) are some of the various virus types employed as vectors in gene therapy .Despite the fact that the viral vectors(6)

Methods of Gene Therapy:

Genetic modification Replacement treatment; Corrective Gene therapy; 2. Physical, chemical, biological; 3. Gene transfer in a particular cell line Other techniques of genetic engineering involve targeting and knocking off specific genes with designed nucleases such as zinc finger nucleases, engineered I-CreI homing endonucleases, or nucleases created from TAL effectors. Somatic gene therapy; germ line gene therapy; 4. Eugenic method (gene insertion).This method is now applied in a number of human clinical.(6)

Vectors for Gene Therapy:

A variety of viruses are employed as vectors for gene therapy.

Retroviruses:

A class of viruses with the ability to copy their RNA genomes in double-stranded DNA.These genome copies are capable of integrating into host cell chromosomes.HIV stands for human immunodeficiency virus.For example, one issue with employing retroviruses for gene therapy is that the virus's genetic material can be randomly inserted into a chromosome or inserted into any

arbitrary location within the host's genome by the enzyme integrase.One of the original genes of the host cell will be disturbed if genetic material is accidentally placed in the middle of it (insertional mutagenesis).The occurrence of uncontrolled cell division, or cancer, can be caused by a gene that regulates cell division.

Recently, efforts have been made to overcome this issue by employing zinc finger nucleases[3] or by including certain sequences, such as the beta-globin locus control region, to direct the location of integration to particular chromosomal Sites .(6)

Adenoviruses:

The virus that causes the common cold is called an adenovirus. A class of viruses with double - stranded DNA genomes that cause infections in the respiratory, gastrointestinal, and ocular systems in humans. (6). Depending on when they express during the viral replicative cycle, adenoviral genes can be classified into three primary groups: early (E1A, E1B, E2, E3, and E4), delayed (IX and IVa2), and the major late transcription unit . The latter is split up into five mRNAs with the same carboxy terminus (L1–L5). Cellular RNA polymerase II transcribes these transcription units, while RNA polymerase III transcribes the viral-associated (VA) RNA. Within each terminal repeat of the virus, there are two identical origins for DNA replication. Proteins from the E1 and E4 sections also aid in effective DNA replication, and the E2 region encodes proteins necessary for replication, such as the viral polymerase. The E3 region's gene products play a role. (2).

Adeno-Associated Viruses:

A class of tiny, single-stranded DNA viruses that can insert their genetic material at a specified location on chromosome 19.(6) AAV is a human parvovirus that is not harmful (see to Reference 56 for a comprehensive analysis of parvovirus biology). Coinfection with helper viruses, such Ad and herpesvirus, can provide the helper functions needed for productive AAV infection.

In addition, AAV can proliferate in cells that have undergone stressful treatments like radiation or genotoxic chemical treatment. The viral DNA can integrate into the host chromosomal genome to create a latent infection if there isn't a conducive environment to enable AAV replication.(2)

CIS and Trans-Acting elements:

Replication-defective vectors always contain a "transfer construct," which carries the gene to be transduced, or "transgene." The transfer construct also carries sequences that are required for the general functioning of the viral genome, such as packaging sequences, repeats for replication, and, when necessary, priming of reverse transcription. These sequences are called cis-acting elements because they must be on the same piece of DNA as the viral genome and the gene of interest. Herpes complex viruses are a class of double-stranded DNA viruses that infect specific cell types, such as neurons. Herpes complex virus type 1 is a common human pathogen that causes cold sores.(6)

Herpes Simplex Viruses:

A class of double- stranded DNA viruses known as herpes simplex viruses targets neurons specifically. Cold sores are commonly caused by the human disease Herpes simplex virus type 1. The class of big DNA viruses known as human herpesviruses has double-stranded genomes that may accommodate vast amounts of foreign DNA. A vector for delivering genes has been created using the herpes simplex virus type 1 (HSV-1). The envelope, tegument, capsid, and viral genome comprise the four components of the 20 nm-diameter HSV-1 virion. The envelope, which is made of the cellular membrane, has about 12 viral glycoproteins that are necessary for the virus to enter the body. Between the capsid and the envelope lies a protein layer called the tegument, which has at least 10 viral proteins in it. These proteins are important in both the activation of immediate early viral gene expression and assembly functions.(2)

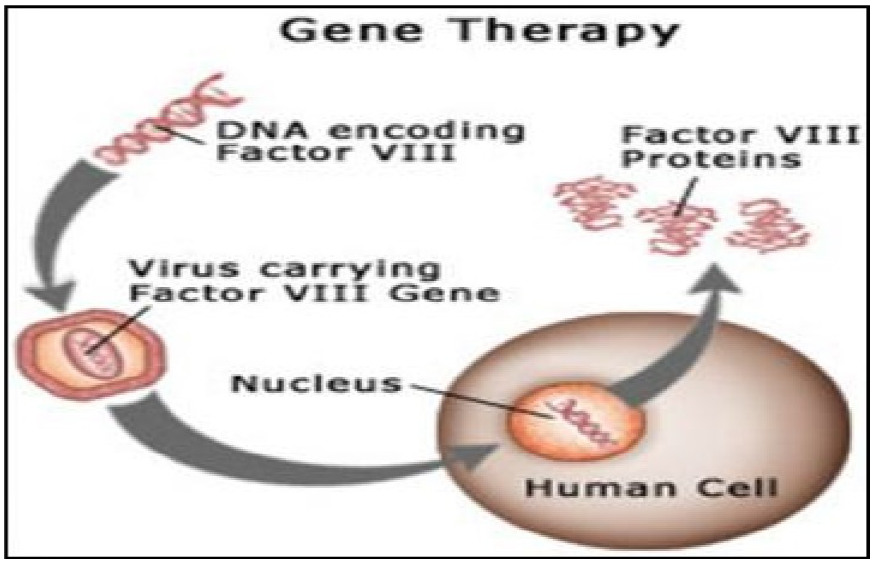

Figure: 2: Viral method of gene therapy.

Non-Viral Methods:

There are a few benefits that non-viral techniques have over viral ones, two of which are easy large-scale production and minimal host immunogenicity. Non-viral approaches used to be at a disadvantage due to low levels of gene expression and transfection, but new developments in vector technology have produced compounds and procedures with transfection efficiencies close to those of viruses. (6)

Ormasil, a silica or silicate modified biologically, is utilized as a non-viral technique. (6)

Methods for Gene Therapy of Cancer:

Viruses
Naked DNA (vector-free)
Liposomes
Protein-DNA complexes
Gene gun
Calcium phosphate precipitation
Electroporation
Intracellular micro injection (1)

Administration

Ex vivo

Cells are removed, genetically modified and transplanted
back into a patient.(1)

In vivo

Direct transfer of genetic material into patient.(1)

Choices of vectors (1)

Viral vectors:
Retrovirus
Adenovirus
Adeno-associated virus
Herpes Simplex Virus (1)

Non-viral vectors (1)

Liposome
DNA–polymer conjugates
Naked DNA (1)

Step-by-step gene therapy:

The patient's bone marrow is extracted and cultured in a lab setting . The desired gene is exposed to the virus that is growing inside the cultured cells. The patient receives the cells back through an injection into a vein following infection and integration of the desired gene into the DNA of the cells. Because the gene is given to the cells while they are outside the patient's body, this procedure is known as ex vivo. Using the in vivo method Liposomes—fatty particles—are used to transmit the desired gene to the patient's inside cells. (1)

Risks Associated with Current Gene therapy:

Viruses can infect many kinds of cells. Viral vectors may modify more than just the target cells. In other words, cancer or other harm could result from the external gene being placed into the wrong spot in the DNA.

There is a chance that some DNA may be injected into germ cells after direct tumor injection, leading to inherited alterations. The virus may spread to other people or the environment; the gene may be overexpressed (toxic); the viral vector may induce inflammation or an immunological response. (1)

Gene delivery in vivo:

Because it avoids the logistical and legal challenges of ex vivo cell-based gene treatments, which call for cell collection, culture, manipulation, and transplantation, targeting organs in vivo is highly appealing. On the other hand, in vivo methods rely on local administration, tissue-specific targeting, and/or target cell-specific gene expression.(3)

Concerns about unintentional germ line change and the possibility of immunological reactions to vector components exist. Some of these obstacles have been surmounted, and promising clinical results from experiments delivering genes to the retinal pigment epithelium of the eye or the liver have opened the door for additional advancements targeting other tissues, such as the brain and muscle.(3)

Gene editing:

Recently, gene-editing technology has become a viable treatment option for a number of illnesses, including as cancer, infectious diseases, and genetic disorders. The exact alteration of the eukaryotic genome for therapeutic purposes has been made possible by the extraordinary advancements in nuclease engineering. Restrictions enzymes known as "gene editing nucleases" have DNA-binding domains that have been modified to cause double-strand breaks (DSBs). Zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease Cas9 (CRISPR/Cas9) are the three main categories of gene-editing nucleases, arranged in chronological sequence of their discovery. (8)

Gene editing in vivo and ex vivo:

Two main approaches are available for the administration of therapeutic gene editing: 1: the direct in vivo distribution of a gene-editing nuclease, and 2: the introduction of ex vivo modified cells containing a gene-editing nuclease . Since the cargo for gene editing typically consists of a plasmid vector or mRNA containing the gene-editing nuclease, appropriate delivery mechanisms for in vivo gene editing include viral vectors and cationic lipid- or polymer-based non-viral vectors. There have occasionally been local injections of bare plasmids into specific tissues, like the eye and muscle. The advantages of gene editing can be more easily applied to a variety of disorders than with other methods because systemic injection of gene-editing nucleases can elicit gene alterations in numerous tissues, depending on the delivery mechanism. (8)

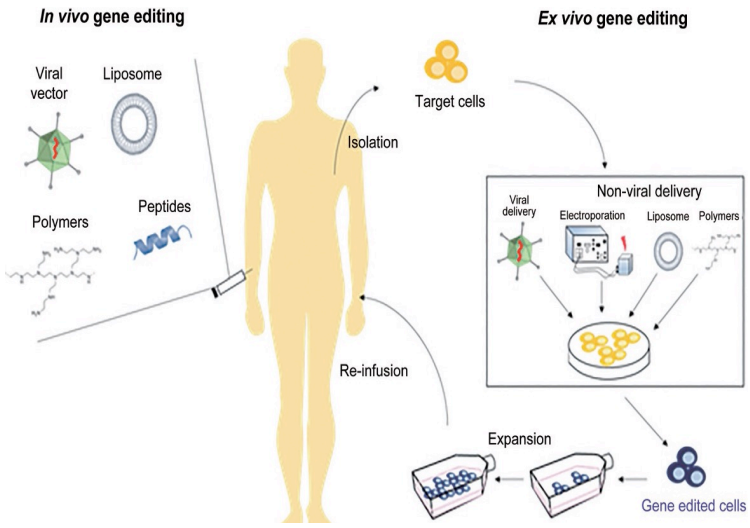

Figure 3: Therapeutic gene-editing strategies.

A schematic depiction of *in vivo* and *ex vivo* gene editing is shown. For *in vivo* gene editing, viral or non-viral vectors carrying nucleases are directly injected into the body. For *ex vivo* gene editing, the target cells are isolated and gene-edited with viral or non-viral vectors carrying nucleases, after which gene-edited cells are expanded and reinfused into the body

Physical Approaches to Improve Delivery:

Electroporation:

This technique transfers DNA across the cell membrane by means of brief, high-voltage bursts. It is believed that this shock creates transient holes in the cell membrane that permit DNA molecules to flow through. Electroporation works with a wide variety of cell types and is generally efficient. Nevertheless, a high incidence of cell death that occurs after electroporation has restricted its application, particularly in therapeutic settings.(6)

Gene Gun:

Another physical technique for transfecting DNA is the use of particle bombardment, sometimes known as the gene gun. This method involves coating DNA with gold particles and loading it into a machine that creates force in order to allow the gold and DNA to enter the cells.For instance, if DNA is incorporated into the genome at the incorrect location, such as a tumor suppressor gene, it may cause a tumor. In clinical studies for patients with X-linked severe combined immunodeficiency (X-SCID), hematopoietic stem cells were transduced with a corrected transgene using a retrovirus; as a result, 3 out of 20 patients developed T cell leukemia.(6)

Sonoporation: Sonoporation introduces DNA into cells by means of ultrasonic frequencies. It is believed that DNA can enter cells through the disruption of the cell membrane caused by the process of sonic cavitation.(6)

Magnetofection:

To bring DNA complexes into touch with a cell monolayer, a magnet is positioned underneath the tissue culture plate after DNA has been complexed to a magnetic particle.(6)

Chemical Approaches to Improve Delivery:

Oligonucleotides:

Synthetic oligonucleotides are used in gene therapy to inactivate the genes responsible for the disease process. This can be accomplished using a variety of techniques. One tactic is to stop the defective gene's transcription by using antisense that is unique to the target gene. Another method involves using tiny RNA molecules known as siRNA to instruct the cell to cleave particular, distinct sequences in the defective gene's mRNA transcript, preventing the incorrect mRNA from being translated and, consequently, the gene's expression.(6)

Lipoplexes and polyplexes:

The DNA needs to be shielded from damage and positively charged in order to enhance the transfer of fresh DNA into the cell. At first, neutral and anionic lipids were employed in the creation of lipoplexes for artificial vectors.(6)

Dendrimers:

A dendrimer is a spherically shaped, heavily branching macromolecule. There are numerous ways to functionalize a particle's surface, and the surface determines a lot of the resulting construct's characteristics. Specifically, a cationic dendrimer—that is, one with a positive surface charge—can be created. Charge complementarity causes the nucleic acid to momentarily bind to the cationic dendrimer when genetic material, such as DNA or RNA, is present. The dendrimer-nucleic acid complex is then absorbed into the cell by endocytosis after it has arrived at its target.(6)

Hybrid approaches:

Since every gene transfer technology has drawbacks, some hybrid approaches that integrate two or more approaches have been developed. One such is a virosome, which combines liposomes with an inactivated influenza or HIV virus . In pulmonary epithelial cells, this has been demonstrated to have more effective gene transfer than liposomal or viral approaches alone. Additional techniques include hybridizing viruses or combining different viral vectors with cationic lipids.(6)

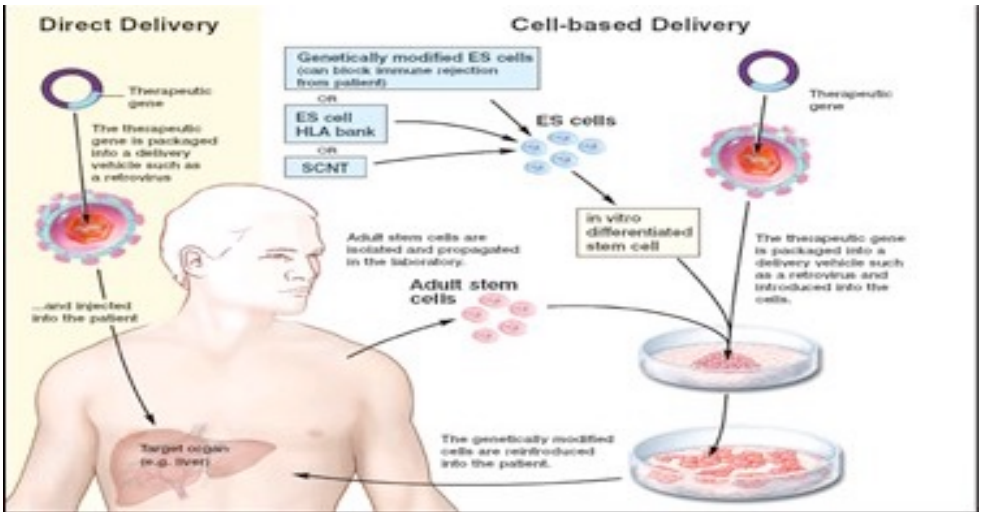

Fig ure 4: Delivery of gene by direct and based methods.

Advantages of gene therapy

In case of 'silence' a gene. In the case of someone with HIV, which had not yet developed into AIDS, scientists could save them the pain and suffering of the disease by using gene therapy to silence' the disease before its onset.

Gene therapy has the potential to eliminate and prevent he-reditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer.

These sceptics would almost certainly choose gene therapy, especially if it was the last hope for them or one of their loved ones – as is the case for many gene therapy patients.(6)

Disadvantages of Gene Therapy

The transient duration of gene therapy.

Immune response: Injecting viruses into cells can cause the body to mount an offensive against the virus. Issues with viral vectors (the vector may regain its capacity to spread illness once it is within the patient).

Multigene disorders: These occur when genetic material does not reach the correct cell or location within the cell's DNA. (6)

The following are some ethical questions about gene therapy:

1. Who determines what characteristics are normal and what is a handicap or disorder?

2. Will only the wealthy get access to gene therapy due to its exorbitant cost?

3. Could the increased use of gene therapy lead to a decrease in societal acceptance of individuals who are different? (6)

4. Is it acceptable for humans to employ gene therapy to improve fundamental human characteristics like height, IQ, or athleticism?(6)

Applications:

Gene therapy is designed with two main goals in mind:

(i) inserting genetic information into the cells to compensate for the abnormal genes, and (ii) producing a beneficial and advantageous protein. If a mutant gene results in the absence or malfunction of an essential protein, gene therapy may be able to restore protein function by introducing a common copy of the gene.

A gene typically cannot function if it is directly put into a specific cell. A vector is a carrier that has been genetically modified to deliver genes rather than genes. Certain viruses have the ability to transfer the gene. Since the cell must be infected for this gene transfer to occur, these viruses are typically

Before gene therapy be used as a specific kind of treatment for illnesses, researchers must overcome a number of technological obstacles. (4)

1. When it comes to Parkinson's illness:

According to The Independent, gene therapy has been shown to be effective in treating Parkinson's disease. Numerous other newspapers have also expressed the hope that

the new therapy, which aims to increase levels of GABA (a brain neurotransmitter that is deficient in Parkinson's patients), offers. 45 patients with serious diseases had tubes leading to the movement-related regions of their brains implanted in their brains as part of a modest study of the method.

One half received an injection of a virus with a gene that would boost the production of GABA. One-half of the group received a safe saline solution. After six months, the movement of individuals receiving gene therapy improved by 23%, which was twice as much as that of those receiving sham surgery.(6)

2. When Alzheimer's illness is present

By employing a novel method of administering medication directly to the brain, researchers have effectively turned off a gene linked to Alzheimer's disease, according to the Daily Mirror. According to the newspaper, researchers have injected medications into mice's brains using "tiny particles called exosomes, which are released by cells."These headlines are the result of a laboratory investigation that was done on mice. The results are noteworthy because they show that exosomes may be utilized to deliver gene therapy to specific brain genes. BACE1, which generates a protein linked to Alzheimer's disease, is one of these genes.(6)

Potential applications of gene therapy

Combining gene therapy with nanotechnology can treat cancer. March 2008. A treatment that targets and kills cancer cells that are difficult to reach is being tested in mice by the London School of Pharmacy. The treatment involves delivering genes packaged in nanoparticles to the cancer cells. Go through the BBC article.

The first gene therapy for genetic blindness has shown promising results in improving sight. April 28, 2008. Results from the world's first clinical trial testing a ground-breaking gene therapy treatment for a form of inherited blindness have been released by UK researchers from the UCL Institute of Ophthalmology and Moorfields Eye Hospital NIHR Biomedical Research Centre.

Two adult patients with a condition affecting myeloid cells, non-lymphocytic white blood cells, are successfully treated with gene therapy. Numerous bone marrow failure syndromes, including acute myeloid leukemia, are examples of the common myeloid diseases. This is the first study demonstrating the potential of gene therapy to treat myeloid system disorders. Check out the groundbreaking international study that suggests gene therapy may be able to treat myeloid blood diseases .

A research team at the University of California, Los Angeles uses liposomes coated in polyethylene glycol (PEG) to deliver genes into the brain. A major accomplishment is the transfer of genes into the brain, as viral vectors are too large to pass across the "blood-brain barrier." This approach may be used to treat Parkinson's.

PROBLEMS:

Gene therapy has received a great deal of interest since the first clinical gene therapy procedure and both the public and corporate sectors have invested a sizable amount of money in it. Additionally, there has been a notable increase in the field's research activities. Numerous research have been conducted using preclinical animal models, and these investigations have produced concept proofs for several possible clinical applications. Additionally, there have been significant advancements in our knowledge of the biology of vectors and in the creation and design of vectors.

Despite the aforementioned points, clinical research has not advanced quickly. Furthermore, a major setback for the science occurred in September of this year when reports of many gene therapy-related deaths surfaced .

An eight-year-old man at the University of Pennsylvania passed away during a research experiment. In this experiment, the hepatic enzyme ornithine decarboxylase was delivered via an Ad5 vector that was modified.

According to a university study, the man's death was brought on by a significant immune response to the administered Ad5 vector.(4)

Conclusion:

Gene therapy is a treatment option for certain genetic disorders. For example, a single trial is

conducted in cases of Parkinson's illness, diabetes, AIDS, hepatitis, melanoma, and Alzheimer's disease. A biotech startup called Neurologix reported that their historic Phase I gene therapy experiment for Parkinson's disease has been successfully concluded .(6) It's evident that a lot of fascinating advancements are occurring in the field of gene therapy. Many of these novel biotech and gene-therapy items may have unidentified hazards at this time, but they also have the potential to provide enormous patient benefits.(1)

Gene therapy is a revolutionary field in medicine that offers hope for individuals affected by a wide range of genetic and acquired diseases. While challenges and ethical considerations remain, ongoing research and clinical trials continue to advance this promising approach, making it a key player in the future of health care.

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