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Gene therapy: A progress in childhood disease

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Abstract

As gene therapy is one of the hottest topics of the new century, it carries the excitement of a cure to most of dis controversy surrounding the altering of human imperfection, and the promise of a type of medical treatment most of cause would never imagine possible

The recent sequence of the human genome combined with the development of massively high throughput genetic analysis technologies is driving unprecedented growth in knowledge of the molecular basis of disease. While this has already had a major function in our diagnostic power, the therapeutic benefits remain largely unrealized.

This review examines progress in the exciting and challenging form of gene therapy, in particular we focus on the treatment of genetic disease in infants and children where the most significant successes have been observed to date, despite the majority of total participants coming from adults. Notably, gene transfer to the haematopoietic compartment has provided the clearest examples of therapeutic benefit, particularly in the context of primary immunodeficiencies. (1)

Material and methods: A thorough search of literature carried out through the National library of medicine (PubMed), SCOPUS, EMBASE databases using different Keywords. All the relevant articles were analyzed according to their importance

And reviewed to evaluate the past present and future perspective of gene therapy. Resalty: With a possibility to eliminate and present AIDS, malignancies, hereditary disorders and it's conceivable cure for cardiac disorders, gene therapy is nothing short of a medical phenomenon. (1)

Keywords: Clinical Trials; Gene Therapy; Genetics; Gene Transfer; Paediatrics

1. Introduction

Gene therapy is a rapidly growing field of medicine in which genes are introduced into the body to treat diseases. Genes control heredity and provide the basic biological code for determining a cell's specific functions.

Gene therapy seeks to provide genes that correct or supplant the disease- controlling functions of cells that are not, in essence, doing their job. Somatic gene therapy introduces therapeutic genes at the tissue or cellular level to treat a specific individual.

Germ-line gene therapy inserts genes into reproductive cells or possibly into embryos to correct genetic defects that could be passed onto future generations. Initially conceived as an approach for treating inherited diseases, like cystic

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fibrosis and Huntington's disease, the scope of potential gene therapies has grown to include treatments for cancers, arthritis, and infectious diseases.

Although gene therapy testing in humans has advanced rapidly, many questions surround its use. For example, some scientists are concerned that the therapeutic genes themselves may cause disease. Others fear that germ-line gene therapy may be used to control human development in ways not connected with disease, like intelligence or appearance.

Gene therapy (also called human gene transfer) is a medical field which focuses on the utilization of the therapeutic delivery of nucleic acid into a patient's cells as a drug to treat disease.

The first attempt at modifying human DNA was performed in 1980 by Martin Cline, hut the first successful nuclear gene transfer in humans, approved by the National Institutes of Health, was performed in May 1989.

The first therapeutic use of gene transfer as well as the first direct insertion of human DNA into the nuclear genome was performed by French Anderson in a trial starting in September 1990. It is thought to be able to cure many genetic disorders or treat them over time

Numerous human geneticists have toyed with the idea of gene transfer for the treatment of inherited diseases. In the last few years that that remarkable advances in recombinant DNA technology and cell biology hava mode it likely that this pipedream will hector come reality and fetuses- gene therapy will not be restricted for the correction of single gene disorders, bear will have applications for many branches of mediate (1)

- **Aim:** Gene Therapy -A Progress in childhood disease.
- **Objective:** The objective of gene therapy studies is to develop ways to treat or prevent disease by altering a person's genes:
- **Correct faulty genes**: Replace a disease-causing gene with a healthy copy, or Inactivate a faulty gene
- **Introduce new genes:** Introduce a new or modified gene into the body to help treat a disease
- **Deliver genetic material**: Use non-toxic gene carrier to deliver foreign genetic material into specific cell types or tissues

Gene therapy is a medical approach that can be used to treat a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia, and AIDS.

The goal of gene therapy for childhood diseases is to treat or prevent disease by correcting the underlying genetic problem. Gene therapy is a promising technology that aims to

- **Replace faulty genes:** Replace a gene that isn't working properly with a healthy gene
- **Add a "good" gene:** Add a "good" gene into a person who has a disease
- **Block a harmful gene:** Remove a harmful gene from a cell

Gene therapy is a rapidly advancing field that offers a shift from traditional treatments by targeting the root cause of genetic diseases. It's a medical approach that allows doctors to treat a disorder by altering a person's genetic makeup instead of using drugs or surgery (3)

2. Literature Review

2.1. Santosh R Patil (December 2010)

As gene therapy is one of the hottest topics of the new century, it carries the excitement of a cure to must af dis cases, the controversy surrounding the altering of human imperfection, and the promise of a type of medical treatment most of us would never imagine possible, a thorough search of literature carried out through the National library of medicine (PubMed), SCOPUS, EMBASE databases using different Keywords. All the relevant articles were analyzed according to their importance. And reviewed to evaluate the past present and future perspective of gene therapy with a possibility to eliminate and prevent AIDS, malignancies, hereditary disorders and it's conceivable cure for cardiac disorders, gene therapy is nothing short of a medical phenomenon.Gene therapy has potential in treatment for most of the diseases (1)

2.2. Samantha L Ginn' and lan E Alexander (June 2011)

The recruit sequencing of the human genome combined with the development of massively high throughput genetic analysts' technologies is driving unprecedented growth in our knowledge of the molecular basis of disease. While this has already had a major impact on our diagnostic power, the therapeutic benefits remain largely unrealized. The rewire examines progress in the exciting and challenging field of gene therapy.

In particular we focus on the treatment of genetic disease in instants and children where the most significant successes have been observed to date, despite the majority of trial participants being adults. Notably, gene transfer to the haematopoietic compartment has provided the clearest examples of therapeutic benefits, particularly in the context of primary errmanomelitencies. The Triumphs and tribulations explored, and the key challenges confronting marcher's as they seem to further advise the finks are defined and discussed. (1)

3. Megha Sahu and Duragkar (May 2012)

Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which will deliver the gene of interest into the target cells. Based on the nature of the viral genome, these gene therapy vectors can be divided into RNA and DNA viral vectors. The majority of RNA virus-based vectors have been derived from simple retroviruses like murine leukemia virus. A major shortcoming of these vectors is that they are not able to transducer non dividing cells. This problem may be overcome by the use of novel retroviral vectors derived from lentiviruses, such as human immunodeficiency virus (HIV), The most commonly used "NA virus vectors are based on adenoviruses and adeno- associated viruses. An example of gene-knockout mediated gene therapy is the knockout of the human CCR5 gene in T-cells in order to control HIV infection [Although the available vector systems are able to deliver genes in vivo into cells, the ideal delivery vehicle has not been found. Thus, the present viral vectors should be used only with great caution in human beings and further progress in vector development is necessary (2).

3.1. Shayla Raj, Rohit Ravinder, Preeti Mishra (June 2021)

The goal of gene- enhanced tissue engineering is to regenerate lost tissue by the local delivery of cells that have been genetically-enhanced to deliver physiologic levels of specific growth factors.

The basis for this approach lies in the presence of a population of progenitor cells that can be induced, under the influence of these growth factors, to differentiate into the specific cells required for tissue regeneration, with guidance from local clues in the wound environment (2)

3.2. Discovery

- Between 1989 and December 2018, over 2,900 clinical trials were conducted, with more than half of them in phase 1.[4]
- As of 2017, Spark Therapeutics' Luxturna (RPE65 mutation- induced blindness) and Novartis' Kymriah (Chimeric antigen receptor T cell therapy) are the FDA's first approved gene therapies to enter the market.

Since that time, drugs such as Novartis Zolgen Sma and Alnylam's Patisiran have also received FDA approval, in addition to other companies' gene therapy drugs. Most of these approaches utilize adeno- associated viruses (AAVs) and lentiviruses for performing gene insertions, in vivo and ex vivo, respectively. ASD/ siRNA approaches such as those conducted by Alnylam and Ionis Pharmaceuticals require non-viral delivery systems, and utilize alternative mechanisms for trafficking to liver rells by way of GalNAc: transporters. The introduction of CRISPR gene editing has opened new doors for its application and utilization in gene therapy.

Solutions to medical hurdles, such as the eradication of latent humans. Immunodeficiency virus (HIV) reservoirs and correction of the mutation that causes sickle cell disease, may soon become a tangible reality.

Not all medical procedures that introduce alterations to a patient's genetic makeup can be considered gene therapy. Bone marrow transplantation and organ transplants in general have been found to introduce foreign DNA into patients. Gene therapy defined by the precision of the is procedure and the intention of direct therapeutic effect (2)

3.3. What are Genes

Genes of heredity nucleotides located in a particular in a particle are the fundamental physical and functional anti gene is an order sense of nuclear position on a particular chromosome that it encodes a specific functional product (i.e., a ptomaine or RNA molecules Ciene is termed as a "biological units' vi heredity liberated from the parents, determine the maintains -like the color of the eyes and calin and texture of the hair.

They also determine things like whether the child will be male or female, the amount of oxygen the blood can carry, and what the 10 will be. Genes are composed of long strands of a molecule called DNA and are located in a single file within the chromosomes.

The genetic message is encoded by subunits of the DNA called nucleotides. There are approximately three billion pairs of nucleotides in the chromosomes of a human cell. Each person's genetic makeup has a unique sequence of nucleotides, and this is what makes us different from one another. Scientists believe that every human 30.000 genes per cell HA emanation or imperfection in any one e of these genes can result in a physical disease.

These mutations can easily disability or shortened life span. These from one generation to another inherited sast like a mother's blond hair or a father's brown eyes.

An experimental procedure aimed at replacing, manipulating, or supplementing nonfunctional or malfunctioning genes with healthy genes. Genes are specific sequences of bases that encode instructions on how to make protein. Although genes get a lot of attention, it's the proteins that perform most life functions and even make up the majority of cellular structures. When genies are altered so that the encoded proteins are unable to carry out their normal functions, genetic disorders can result (2)

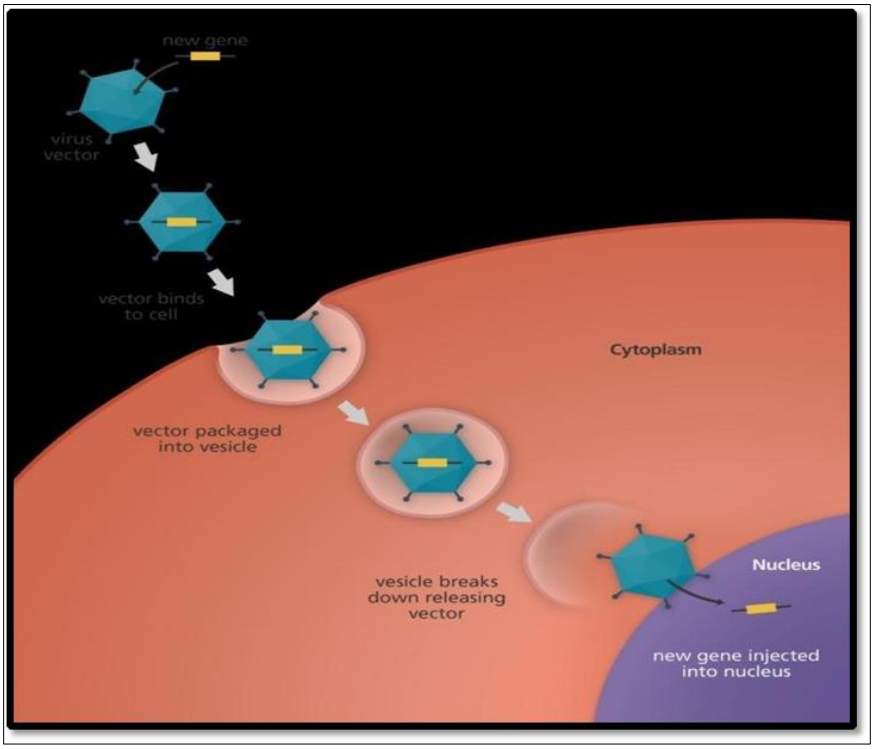

Figure 1 Process of Gene Therapy

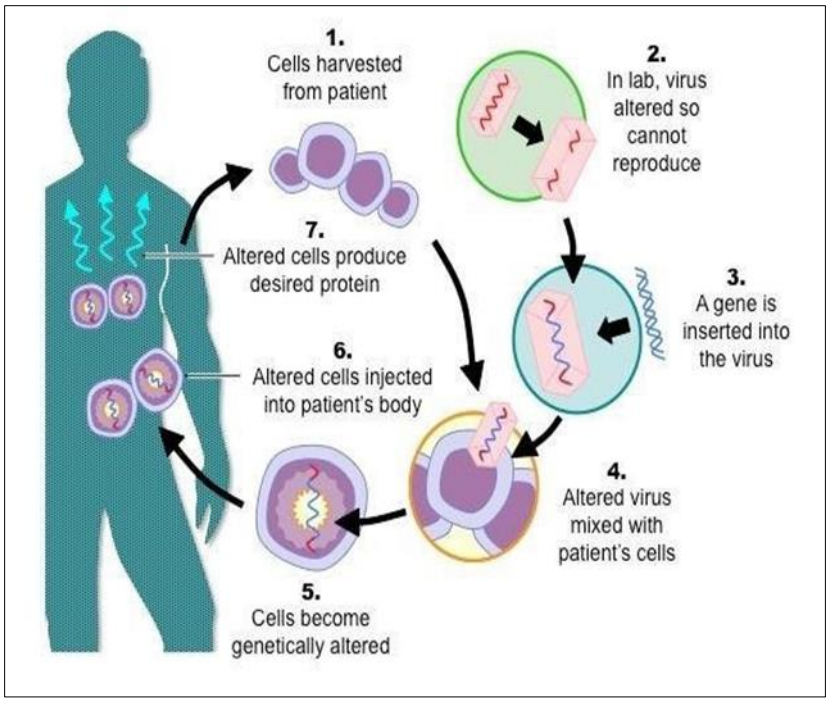

Figure 2 Gene Therapy

3.3.1. Research may use one of several approaches for correcting Faulty Genes

- A normal gene may be inserted into a nonspecific location within to replace a a nonfunctional nonfans gene
- This approach is most genome to an abnormal gone could be swapped for a nominal gene through homologous recombination.
- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal fraction
- The regulation the degree in which a gene is turned on or off) of a particular gene could be altered

3.3.2. Two Types of gene therapy

Somatic gene therapy

Involves introducing a "good" gene into targeted oils with the aid result of treating the patient -but not the patient's future children because these genes do not get passed along to offspring.

In other words, even though some of the e patients' genes may treat a disease, the remains of the same disease will affect the patient's children. This is the form of gene therapy that is being done at most genetics laboratories throughout the world. (2)

3.3.3. Germline gene therapy

entail injecting foreign genes into fertile eggs or in spams producing cells, which will then pass any genetic changes to future generations as well.

However, although it has potential for preventing inherited disease, this form of gene therapy is extremely controversial and currently very little research is being done in this area, both for technical and ethical reasons.

3.3.4. Methods for Gene therapy

Physical

- Direct injection of DNA
- Liposome-mediated DNA transfer
- Calcium phosphate transfection

- Electroporation

Retrovirus vectors.

- Other viral vectors
- Targeted gene transfer via receptors
- Artificial Chromosomes
- Site e directed recombination
- Activation of genes related function.

In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-massing gene. Carrier molecule called a vector is used to deliver the therapeutic gene to the patient's target cells.

Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of interpolating and delivering their genes to human cells in a pathogenic manner.

Scientists have begun to take advantage of this capability and manipulate the virus genome to remove dissuasiveness and insert therapeutic genes Some of the different types of causes used as gene therapy vectors.

3.4. Retroviruses

A class of viruses that create double stranded copies of their R These INA genomes. These copies of its genome are integrated into the chromosomes of host cells. Human Immunodeficiency Vines (HIV) is a retrovirus.

3.5. Adenoviruses

A class of viruses with double-stranded DNA genomes that cause restructure, intestinal, and eye infectious humans. The virus that causes the common cold is an adenovirus.

3.6. Adeno-associated viruses

Class of small, single stranded DNA viruses that insert their genetic material specific site Chromosome.

3.7. Herpes simplex viruses

Closes of double-stranded DNA viruses that infect a particular cell type, neuron. Herpes simplex virus type 1 is a common human pathogen that causes cold sores. (6)

3.7.1. Target cell for Gene Therapy

- Peripheral blood lymphocyte
- Hematopoietic stem cells

3.7.2. Fibroblasts

Hepatocytes

- Keratinocytes
- Skeletal muscle myoblast
- Airway epithelial cells
- Vascular endothelial cells
- Tumor cells.

3.7.3. Factors Inhibiting Gene Therapy

Short lived nature of gene therapy Before gene therapy can become a permanent cure for any condition, the therapeutic the DNA introduced imo target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable Problems with migrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gone therapy from achieving any long-term benefits. Patients will have to undented multiple rounds of gene therapy. [7]

3.8. Immune response

Anytime a foreign object is introduced in Human t the system is designed the risk of stimulating the immune system to attack the pinto way that redacts gene therapy effectiveness is always a potential risk. Furthermore, the immune system's enhanced response to invaders it has seen before makes it difficult for gene therapy to be repeated in patients

3.9. Problems with viral vectors

Viruses, while the carrier of choice in most gene therapy studies, present a variety of potential to the patient-covicity, immune and inflammatory responses, und gene control and targeting asses. In addition, there is always the fear that tise viral vector, once inside the patient, may recover Its ability to ensure disease.

3.10. Multigene disorder

Conditions or disorders that arise from mutation in a single gene are the best candidates for gene therapy Unfortunately, some of the most commonly occurring disorders, such Disease, high pressure. Alzheimer disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes. N multifactorial disorders such as these would be Multigene or be especially difficult to treat effectively using gene therapy.

3.10.1. Application of Gene therapy

Gene therapy is likely to have the greatest success with diseases that are caused by single gene defects. By the end of 1993, gene therapy hand been approved for use on such disease severe combined immune deficiency.familial hypercholes cool to date is aimed toward the treatment of cancer, a few are also targeted a, cystic fibrosis, and Gaucher's disease

Most protoowards AIDS. Numerous disorders are discussed as candidates for gene therapy.

Parkinson's and Alzheimer's disease, arthritis and heart disease. The human Genome project, an ongoing effort to identify the location of all the genes in the human genome continue to identify genetic diseases. (8)

- Eve Nichols describes the criteria for selection of disease for human gene therapy disease is a morale, Life-threatening disease.
- Organ, tissue and cell types affected by the disease have been identified
- The normal counterpart of the defective gene has been isolated and cloned
- The normal gene con be introduced into a substantial anh fraction of the cells from the affected tissue, or that introduction of the gone into the available target tissue, such as bone marrow, will somehow alter the disease process in the tissue affected by the disease
- The gene can be expected adequately (it will direct the production of enough normal protein to make a difference
- Techniques are available to verify the safety of the procedure

3.10.2. Advantages of Gene Therapy

- Germ-line gene therapy offers a true cure, and not simply palliative or symptomatic treatment
- Germ-line gene therapy may be the only effective way of addressing some genetic diseases
- By preventing the transmission of disease genes, the expense and risk of somatic cell therapy for multiple generations is avoided (9)
- Medicine should respond to the reproductive health needs of prospective parents at risk for transmitting serious genetic diseases
- The scientific community has a right to free inquiry, within the bounds of acceptable human research.

3.11. Disadvantages of Gene therapy

- Germ-line gene therapy experiments would involve too much scientific uncertainty and clinical risks, and the long-term effects of such therapy are unknown.
- Such gene therapy would open the door to attempts at altering human traits not associated with disease, which could exacerbate. Problems of social discrimination,
- As germ-line gene therapy involves research on early embryos and affects their offspring, such research essentially creates nations of unconsulting research subjects.
- Gene therapy is very expensive, and will never be cost effective enough to merit high social priority.

- Germ-line gene therapy would violate the rights of subsequent generations to inherit a genetic endowment that has not been intentionally modified.
- The ethical issues posed by both somatic and germ-line gene three pies are international in scope. The documents listed below serve to demonstrate the variety of reactions to gene therapy, and to illuminate the complexity of this continuing public debate (10)

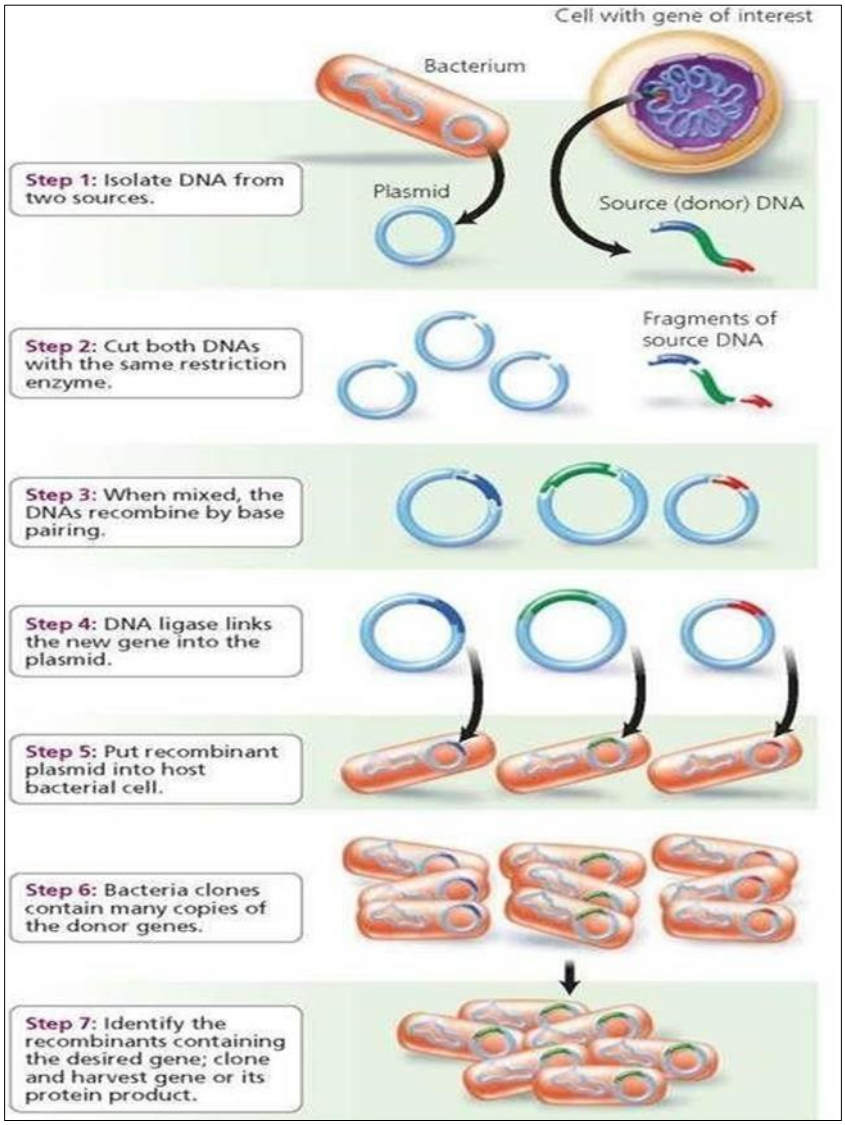

Figure 3 Gene Working

3.12. Ethical issues surrounding gene therapy

- Because gene therapy involves making changes to the body's set of basic instructions, it raises many unique ethical concerns. The ethical questions surrounding gene therapy include
- How can "good" and "bad" uses of gene therapy be distinguished? Who decides which traits are normal and which constitute a disability or disorder?
- Will the high costs of gene therapy make it available only to the wealthy? Could the widespread use of gene therapy make society less
- Accepting people who are different? Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence, or athletic ability? Is Gene Therapy Safe
- 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 unpredicted able,

however, medical researchers, institutions, and regulatory agencies are working to ensure that gene therapy research is as safe as possible. (11)

3.13. Treatment Modalities Using Gene Therapy

3.13.1. Mucocutaneous gene therapy

It offers exciting new treatment modalities for skin and mucosal lesions. Transient expression of naked plasmid DNA could be used as a local treatment of various skin and mucosal lesions where the corresponding gene product (protein) has therapeutic or immunization potential. Ulrich R. Hengge et al analyzed the time course, magnitude, and histologic expression of the indicator plasmid DNA (PCMVB-Gal) in mucosal epithelium and papilloma lesions.

Upon direct injection of naked plasmid DNA (20 μg) into oral mucosa, expression occurred at high local concentrations, up to 35-fold higher than in comparable injections into the epidermis. Due to the accelerated turnover of mucosal epithelium B-galactosidase positive epithelial cells were detected in the basal and suprabasal layers as early as 3 h after injection, whereas only the most superficial mucosal layers demonstrated Galactosidase staining at 24 h post-injection. These biologic characteristics need to be taken into consideration when clinical applications of expressing naked plasmid DNA in epithelial tissues are considered (14)

3.13.2. Herpes viral infection.

Herpes viruses are a diverse family of large DNA viruses, all of which have the capacity to establish lifelong latent infection. Delivery of reporter genes in vitro and in vivo has been demonstrated using a variety of replication competent and replication defective vectors and significant physiological modification in the CNS has been achieved by HSV-mediated gene delivery.

3.13.3. Epstein-Barr virus diseases

Is a herpes virus that infects the majority of the individuals and persists in an asymptomatic state by a combination of chronic replication in the mucosa and latency in peripheral blood B cells. These EBV infected B cells are highly immunogenic and normally susceptible to cytotoxic T lymphocytes. By gene therapy genetic modification of lymphocytes could be a potential treatment for these diseases (13)

3.13.4. Hemoglobinopathies-

Thalassemia Gene therapy for thalassemia requires replacement with a normal functional B-globin gene and in some cases with y-globin gene

3.13.5. Haemophilia A and B

Are relatively rare, X-linked inherited bleeding disorders which are life threatening to the patients unless treated by regular injections of factors VIII and IX respectively.

Gene therapy offers the prospect of a cure for the disease, thus potentially freeing patients from the existing regimens of regular intrave- nous injections of proteins and risk of infection by contaminating viruses. Although gene therapy is very attractive to the patients and clinicians, in practice, preclinical experiments in animal models suggests that it may be difficult to obtain adequate therapeutic levels of either factor VIII or IX for long periods in patients unless improved can be devised

3.13.6. Cystic Fibrosis

Is a common severe autosomal recessive genetic disease which is Caused by dysfunction of epithelial cell surface CAMP activated C channel The effects of this disfunction are pleiotropic but the human morbidity results from the effects in the respiratory epithelium Gene therapy is an attractive possible treatment, the gene required is well characterized and only low-level expression is required (12)

Of all the clinical trials, all the vector system proposed for CF gene therapy, adeno vectors are the most advanced with the results of many clinical trials already published

3.13.7. HIV Infection

The genetic approach to HIV infection is still very young and a number of different stages in the viral life cycle are being studied as targets for gene therapy, using a wide variety of modalities for gene delivery

Several gene therapy protocols for AIDS have been approved. Elimination of virus from an infected person is an unlikely eventuality, therefore the aim must be to maintain the virus in its latent period as long as possible and to protect uninfected cells from viral infection and perhaps to enhance the immune response against virus.

These methodologies are particularly amenable to a gene therapy approach and it seems likely that in future the combination therapy including both pharmacological agents and gene therapy agents, will be used in concert to minimize this spread of the HIV within an infected individual and thus prolong their disease-free life. A gene therapy-based vaccine is also a serious possibility. (17)

3.13.8. Cancer

Several approaches to cancer therapy are being explored

- Immune responses to tumors are being enhanced
- Genes are being inserted into tumor cells to evoke cell suicide
- Finally, methods are being developed to modify tumor suppressor or anti-oncogenes.

The primary target for stimulating an immune response to a tumor is major histocompatibility complex (MHC) class1-restricted tumor specific cytotoxic (CDS) T cell.

3.13.9. Two criteria must be met

- The CDS T cell receptors must be occupied by an MHC class 1 peptide complex
- A helper T cell must be activated to secrete cytokines, which acts on CD 8 T cell

This second signal could be bypassed by inducing CD & cell to produce their own cytokines.Cell suicide involves insertion of Herpes simple's virus thymidine kinase (HSV-TK) gene. Tumor suppressor genes are being inserted into human tumors. One protocol involves inserting a normal p53 gene into non-small cell lung carcinomas that are p53 defective

In another, antisense DNA is injected to try to suppress the activity of activated oncogenes, in this case k-ras in lung carcinoma.

3.13.10. Gene Therapy and viral vaccination

Live viral vaccines have had a major impact on the incidence of acute viral infections world-wide. Virus infections recognized as future vaccine targets will require a modified approach based on the detailed understanding of the immunobiology of specific infections combined with the application of the new technologies designed to generate specific and appropriate protective immunity. A similar vector technology directed at in vivo gene delivery is currently being exploited for both gene therapy and vaccination. The induction of an immune response to an expressed transgene represents potential hazards for a gene therapy protocol but is the object of a vaccine strategy. In Vivo gene delivery using (16)

Replication-competent or replication-deficient viral vector systems and by direct transfer of naked DNA can generate an effective humoral, secretary and cell mediated immune response to expressed transgenes

3.13.11. Also, Gene therapy is a serious consideration in many diseases caused by Virus, Bacteria and other microorganisms.

Edge to provide treatment and/or cures Gene therapy's potential to revolutionize medicine in the future is exciting, and its expectations for curing and preventing childhood diseases are encouraging. One day it may be possible to treat an child in utero for a genetic disease even before it comes in to this world

Scientists are hoping the mapping of the human genome will lead the way toward cures for many diseases and that the successes of current clinical trials will create new opportunities and challenges. For now, however, it's a wait-and-see situation, calling for cautious optimism.

3.14. Definition & History

Gene therapy is a promising treatment for several inherited or acquired hematologic disorders. Gene therapy involves the introduction of a functional gene to replace a mutated gene or a therapeutic gene to provide a missing or defective

Protein to the organism. In some cases, the patient's blood cells are removed and special, targeted cells such as hematopoietic stem cells (HSCs) are selected for engineering and the therapeutic genes are introduced into a vector and delivered into the targeted cells.

These targeted, gene- modified cells are reinfused back into the patient. Because this method modifies cells outside the patient's body, it is called ex vivo gene therapy [22]. By contrast in vivo gene

Therapy describes the therapeutic gene-containing vectors. Being directly injected into the patient [23]. In the in vivo case, the gene is expressed, producing a therapeutic protein for treatment.

Theoretically, if the gene-modified cells are long-lived and able to expand inside the body, a single gene therapy can be sufficient to provide a lifelong therapeutic effect. Current gene therapy technologies have reached the point that many types of single-gene hematologic deficiency diseases can be permanently corrected, for example, X- linked severe combined immunodeficiency (X-SCID) and adenosine deaminase deficiency severe combined. Immunodeficiency (ADA-SCID). (18)

3.14.1. Modern Concept of Gene –

Genes are a unit of inheritance. A gene is a segment of DNA that provides instructions for synthesis of a specific protein or a particular type of RNA. It may be defined as a segment of DNA which is responsible for inheritance and expression of a particular character. Seymour Benzer (19995) introduced the term, cristron, muton and recon.

Cistron

- (Unit of function) It is responsible for expression of a trait. It is a segment of DNA having information for synthesis of a particular protein or RNA. It can be several hundred bp (base pairs) long.

Muton

- (Unit of mutation). It consists of a few nucleotides, (one to few bp long). It is a segment of DNA that can undergo mutation.

Recon

- (Unit of recombination). It is a segment of DNA that is particular on recombination through crossing over during meiosis. It consists of a few to many base pairs.

3.15. Gene Expression and Gene Regulation

We know that in a living cell a gene expresses itself and as a result, either a structural protein or an active protein e.g. enzyme or RNA is produced. All these are required for different metabolic activities. However, gene expression can be controlled or regulated at various levels such as transcriptional or post transcriptional or translational level. Here we are going to discuss the transcriptional regulation of gene expression. Usually, small extracellular or intracellular metabolites trigger either initiation or inhibition of gene expression. The clusters of genes with related functions are called operons. They usually transcribe single mRNA molecules. (21)

3.16. Structure of the Operon

- Each operon is a unit of gene expression and regulation. Which includes the structural genes and their control elements (promoters and operators).
- The structural genes. Code for protein, t RNA and t RNA required by the cell. Promotes are signal sequences in DNA that start RNA synthesis.
- These are the sites where the RNA polymerase is bound during transcription. The operators are present between the promoters and structural genes.
- The repressor protein binds to the operator region of the operon. Regulatory genes are responsible for formation of repressors which interact with operators.

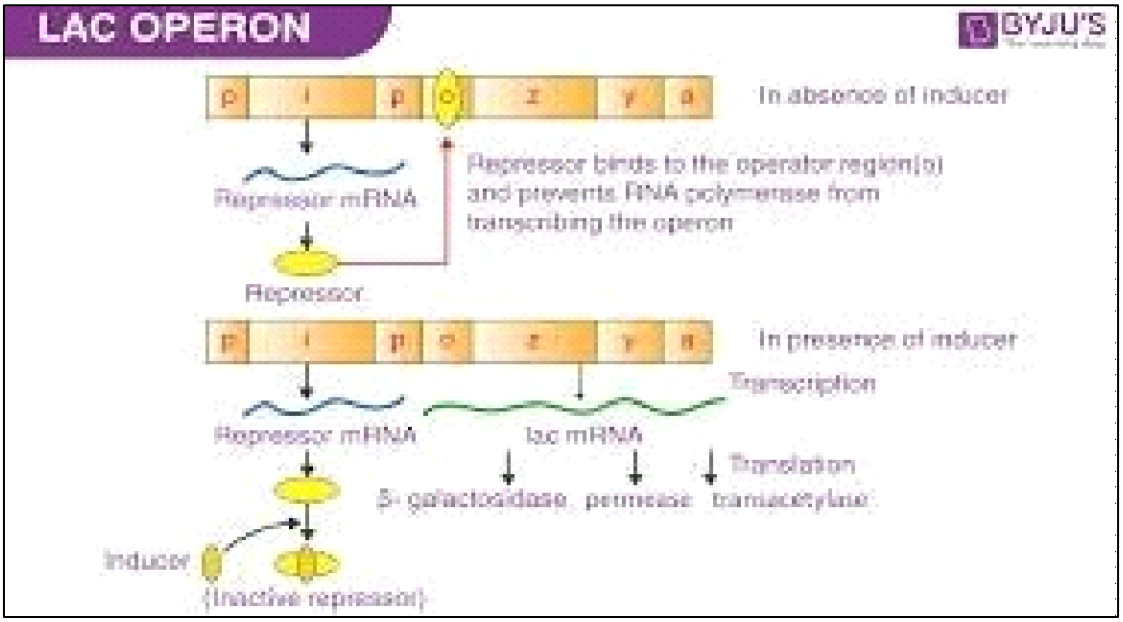

Figure 4 Lac Operon

3.17. The Lac (Lactose) Operon

The metabolism of Lactose in cells requires three enzymes – permease, B-galactosidase and transacetylase. The enzyme permease is needed for entry of lactose in the cell, B galactosidase brings about hydrolysis of lactose into glucose and galactose, while transacetylase transfer acetyl group from acetyl Co 'A' to B galactosidase.The lac operon has promoter sites (p), regulatory site (i) and operator site (o). Besides this it has three structural genes namely z, y and a. The 'z' gene codes for B-galactosidase, 'y' gene codes for permease and 'a' gene for transacetylase.

3.17.1. Francois Jacob and J. Monod

- proposed the clinical model of Lac operon which can properly explain gene expression and regulation in E. coli. In lac operon a polycistronic structural gene is regulated by common promoter and regulatory genes.
- When the cell is using its normal energy source. Glucose, the 'I gene transcribes a repressor m RNA, after translation of which, a repressor protein is produced. It binds the operator region of the operon and prevents the RNA polymerase from transcribing the operon.
- As a result, ß - galactosidase is not produced. In absence of glucose, if lactose is available as an energy source for the bacteria, then following events occur in the cell. The lactose enters the cell
- As a result of the activity of permease enzymes. It acts as an inducer and interacts with the repository to inactive it.
- As the repressor is inactivated, the RNA polymerase can bind itself to the operator site and transcribe the operon to produce lac m RNA which enables formation of all the required three enzymes needed for lactose metabolism. This regulation of lac operon by the repressor is an example of negative control of transcription initiation.
- The lactose operon is a set of genes in bacteria that are responsible for the uptake and metabolism of lactose.The lactose operon is a group of genes in bacteria, such as E. coli, that encode proteins for using lactose as an energy source.
- The lactose operon is only expressed when lactose is present and glucose is absent. The lac operon is regulated by two proteins: the lac repressor and the global activator protein, The lac repressor acts as a lactose sensor and binds to the operator, preventing transcription when lactose is not available. Crp binds to the promoter and stimulates RNA polymerase to bind when there is no glucose (20)

Objective

Gene therapy is used to correct defective genes in order to cure a disease or help your body better fight disease. Researchers are investigating several ways to do this, including: 1. Replacing mutated genes: Some cells become diseased because certain genes work incorrectly or no longer work at all. Replacing the defective genes may help treat certain diseases. For instance, a gene called p53 normally prevents tumor growth. Several types of cancer have been linked to problems with the p53 gene. If doctors could replace the defective p53 gene that might trigger the cancer cells to die. (21)

Fixing mutated genes: Mutated genes that cause disease could be turned off so that.They no longer promote disease, or healthy genes that help prevent disease could be turned on so that they could inhibit the disease. (21)

Risks: Gene therapy has some potential risks. A gene can't easily be inserted directly into your cells. Rather, it usually has to be Delivered using a carrier, called a vector. The most common gene therapy vectors are viruses because they can recognize certain cells and carry genetic material into the cells' genes. Researchers remove the original disease-causing genes from the viruses, replacing them with the genes needed to stop Disease. (21)

4. This technique presents the following risks

4.1. Unwanted immune system reaction

4.1.1. Your body's immune system may see the

newly introduced viruses as intruders and attack them. This may cause inflammation and, in severe cases, organ failure

4.2. Targeting the wrong cells

Because viruses can affect more than one type of cells, it's possible that the altered viruses may infect additional cells, not just the targeted cells containing mutated genes. If this happens, healthy cells may be damaged, causing other illness or diseases, such as cancer (21)

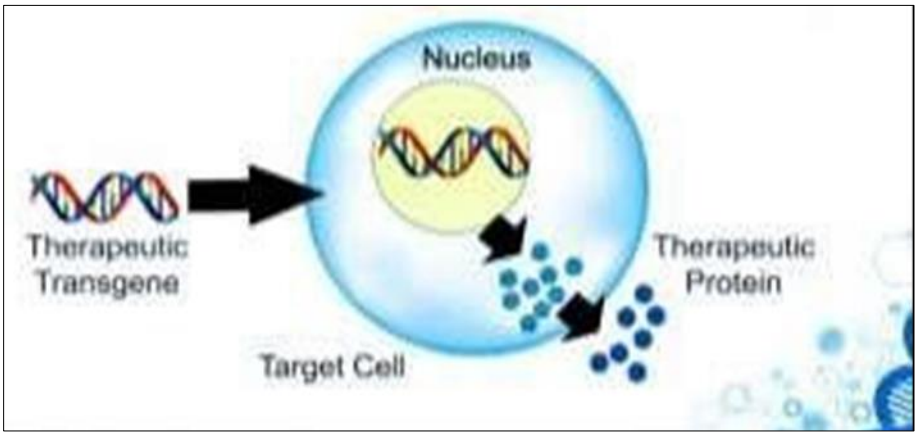

Figure 5 Target cell

4.3. Infection caused by the virus

It's possible that once introduced into the body, the viruses may recover their original ability to cause disease

Viruses aren't the only vectors that can be used to carry altered genes into your body's cells. Other vectors being studied in clinical trials include

4.3.1. Stem cells

Stem cells are the cells from which all other cells in your body are created. For gene therapy, stem cells can be trained in a lab to become cells that can help fight disease.

4.3.2. Liposomes.

These fatty particles have the ability to carry the new. Therapeutic genes to the target cells and pass the genes into your cell's DNA.

Several significant barriers stand in the way of gene therapy becoming a reliable form of treatment, including

- Finding a reliable way to get genetic material into cells
- Targeting the correct cells
- Reducing the risk of side effects

Gene therapy continues to be a very important and active area of research aimed at developing new, effective treatments for a variety of diseases. Vector in gene therapy (21)

The delivery of DNA into cells can be accomplished by multiple methods The two major classes are recombinant viruses (sometimes called biological nanoparticles or viral vectors) and naked DNA or DNA complexes (non-viral methods)

4.3.3. Viral vectors

In order to replicate, viruses introduce their genetic material into the host cell, tricking the hort's cellular machinery intuosing it as blueprints for viral protects Retroviruses go a stage further by having their genetic material copied into the genome of the host cell. Scientists exploit this by restituting a virus's genetic material with therapeutic DNA (The term DNA' may be an oversimplification, as some viruses contain DNA, and gene therapy could take this form as well A number of viruses have been used for human gene therapy Including retroviruses, adenoviruses, herpes simplex vaccinia, and adeno-associated virus Like the genetic material (DNA or RNA) in viruses, therapeutic DNA can be designed to simply serve as a temporary blueprint that if degraded naturally or (at least theoretically) to enter the host's genome, becoming a permanent part of the host's DNA in infected cells.(22)

4.3.4. Non-viral vector

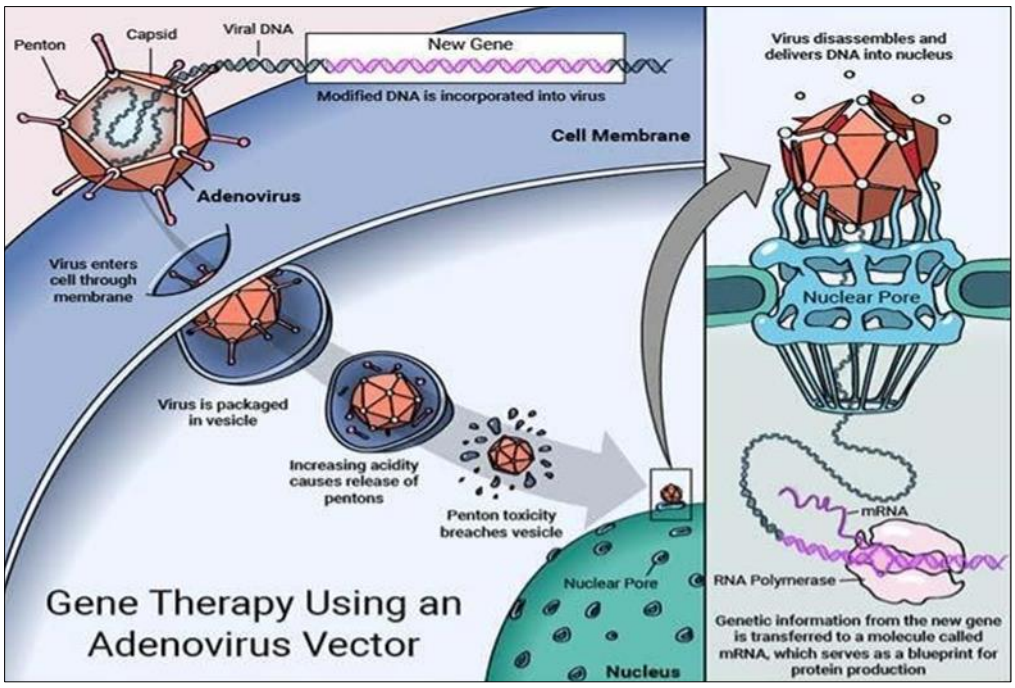

Figure 6 Gene Therapy

Non-viral methods present certain advantages over viral methods, such as large-scale production and low host Immunogenicity However, non-viral methods initially produced lower levels of transfection and gene expression, and thus lower therapeutic efficacy. Newer technologies offer promise of solving these problems, with the advent of Increased cell-specific targeting and subcellular trafficking control Methods for non-viral gene therapy include the injection of naked DNA, electroporation, the gene gun, incorporation, magnetofection, the use of oligonucleotides,

lipoblasts, dendrites, and inorganic nanoparticles. More recent approaches such as those performed by companies such as Ligand, offer the possibility of creating cell-specific targeting technologies for a variety of gene therapy modalities, including RNA, DNA and gene editing tools such as CRISPR. (22)

4.3.5. Challenges of This Type of Therapy

Finding the best delivery system for Transporting normal CFTR genes is only one challenge that scientists m must solve to develop an effective treatment for cystic fibrosis Scientists must also:

- Determine the lifespan of the affected lung cells
- Identify the "parents' cells" that produce CPTR cells
- Find out how long treatment should last and how often it needs to be repeated.

The first CF gene therapy experiments have involved lung cells because these cells are readily accessible and because lung damage is the most common life-threatening problem in CF patients. However, scientists hope that the technologies being developed for long cells will be adapted to treat other organs affected by cystic fibrosis (23)

4.4. Gene therapy: Progress in Childhood Disease

4.4.1. Simple in Theory, Complex in Practice

The basic concept of gene therapy is disarmingly simple: if a given disease is caused by a faulty gene, then repairing, replace ing of mollifying the faulty gene should cure the disease of This conceptual simplicity influenced early expectations that Stevenses in gene therapy would come easily, when in reality therapeutic benefit has been hard won and reported in only a small minority of trials.

The central challenges are to relieve gene transfer to a sufficiently large number of the cells of the required histological type to center therapeutic benefit and to ensure appropriately durable effect. These challenges are disease specific and dependent upon the molecular basis of the disease, location and biology of the target cell population, and availability of suitable gene delivery technology. The pre- dominant strategy used in most clinical trial to date involves simple genes

In addition, which is suitable for recessive genetic diseases and for conferring novel functions on cells, such as drug resistance.

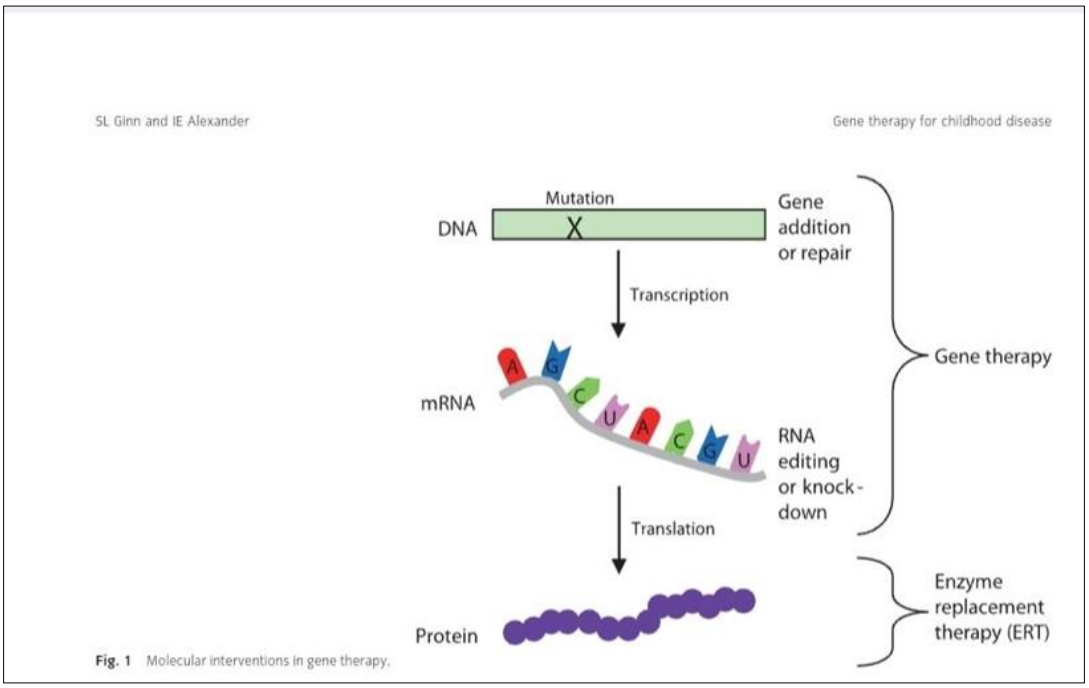

Figure 7 Gene Mutation

In contrast, dominant genetic diseases necessitate that the mutant gene be either repaired by homologous recombination or functionally blocked by mechanisms such as RNA Interference (RNA-mediated destruction of the mutant IRNA Specific destruction of mRNA species can also be used for application such as antiviral therapy. Another developing strategy involves RNA editing, with an exciting example being synthetic oligonucleotide induced exon skipping," which is being trialed for Duchene muscular dystrophy

While the recessive nature of CF Allows phenotype correction by a simple gene addition strategy, correction of the respiratory manifestations will require efficient Gene delivery to airway epithelial cells, which not only necessitate correction in situ but also undergo shedding with epithelial Turnover. Even assuming efficient gene delivery, a durable effect That would avoid the need for repeated therapy necessitates Stable genetic modification of airway epithelial progenitor cells Near the basement membrane, a challenge that lies beyond Contemporary technology (23)

5. Gene Delivery

Gene delivery has been described as the Achilles heel of gene therapy and remains a major focus for developments within the field. Existing technologies can be divided into two broad categories, non-viral for physicochemical and viral, with the latter being most highly developed and broadly exploited. Vertices have evolved to efficiently deliver their genomes in host cells; advent recombinant DNA technology has allowed the engineering of replication of defective recombinant viruses vectors) that lack pathogenicity associated with the parent virus, and obtain favorable aspects of viral biology.

For example, this may include the capacity to specifically deliver genes to a particular cell type, such as a neuron by axoplasmic transport, or to integrate into the host cell genuine, a necessary attribute for stable genetic modification of target well population s, such as haematopoietic stem cells (HSCs), which undergo Vigorous division.

The choice of vector is therefore dependent on the proposed application. Examples in clinical use include adenoviral, retroviral, adeno associated viral LAVI and lentiviral vectors, with each offering distinct strengths and Latinos. Ongoing research is focusing on improving the efficiency, specificity and safety of gene transfer systems and the development of strategies to avoid unwanted immunological responses. (24)

5.1. Of Mice and Men

A Prerequisite for successful gene therapy is the availability of animal models that faithfully recapitulate human disease for preclinical testing. In Almost all cases, the model of choice is the mouse because of the relative ease with which its genome can be manipulated, availability of disease models and relatively low cost. Excitingly, successful phenotype correction by gene therapy in mouse models of human disease is being frequently reported in the biomedical literature.

In 2010 alone there were over 30 publications reporting successful phenotype correction of mice with discuses including mucopolysaccharide- sis type 1, propionic acidaemia, Haemophilia Band Pomp disease. Up to the present time, however, these successes have rarely been replicated in large animal models or human clinical trials. There are a number of reasons for this that include species-specific differences in vector biology and tropism, immune response to the vector or encoded gene product and additional challenges imposed by the sheer size difference of mice and humans.

The average child, for example, is approximately 1000-fold bigger than a mouse and therefore demands a correspondingly greater number of cells to be successfully gene modified. For many conditions this places gene therapy beyond the reach of contemporary gene delivery technologies. Animal modern also provides valuable safety data to assist in risk assessment and to meet regulatory requirements. These models, however, do not always predict phenotypic outcomes or adverse effects subsequently observed in humans. Pleasingly, the extensive successes in mice are beginning to be realized in patients. (26)

5.2. Clinical Successes in Infants and Children

Gene therapy has been successful in treating a variety of childhood diseases, including:

5.2.1. Severe combined immune deficiency (SCID)

The first clinical success of gene therapy, SCID is a life-threatening condition that causes infants to be susceptible to infections. Gene therapy has been highly successful for SCIDX1 infants, especially when enough transduced hematopoietic stem cells (HSCs) are engrafted.

5.2.2. B-cell acute lymphoblastic leukemia (B-ALL)

Chimeric antigen receptor (CAR) T cells targeting CD19 have been successful in inducing complete remissions in refractory patients.

5.2.3. Sickle cell disease

A 13-year-old patient with sickle cell disease who did not respond to other treatments was successfully treated with LV-mediated HSC gene transfer. The patient achieved 47% βglobin expression and no longer required red blood cell transfusions.

5.2.4. Limbal stem-cell deficiency

- A method has been developed to create a curative patch for limbal stem-cell deficiency in a damaged eye.
- Gene therapy may be more successful in newborns than in older children or adults because of the minimal adverse effect of the underlying disease on newborn cells, their relatively small size, and the large amount of future growth.
- Gene therapy involves using genes to fight or prevent diseases. This can be done by replacing a gene that isn't working properly, adding a "good" gene, or blocking a gene that is causing a problem.

5.3. Clinical Ethics

Gene therapy raises many ethical concerns, including:

5.3.1. Informed consent

Patients and their families should be fully informed about the potential benefits, risks, and uncertainties of gene therapy. They should also be aware of the ethical nuances of the treatment and how it interacts with their disease.

5.3.2. Confidentiality

Patients should be able to provide their physicians with information in a confidential manner.

5.3.3. Access

Gene therapy could widen the treatment gap between high-resource and low-resource settings.

6. Discrimination

- Gene therapy could make society less accepting of people who are different.
- Unintended consequences
- Gene therapy could have unintended consequences, such as the DNA being delivered to the wrong cells or the changes not carrying over to new cells.
- Changing reproductive cells
- Changing reproductive cells could have undesirable effects that are passed on to the patient's children.

6.1. Other ethical issues include

How to distinguish between "good" and "bad" uses of gene Therapy Who decides which traits are normal and which are disorders Whether gene therapy should be used to enhance basic human traits.

6.2. Future direction

Gene therapy is a promising treatment for childhood diseases, and the future of gene therapy in this field looks promising:

6.3. New delivery methods

Customized viral vectors and non-viral nanoparticle systems are being developed to improve gene therapy delivery. These new methods may help overcome the challenges that have limited progress in the past.

6.4. Gene editing strategies

Gene editing strategies like CRISPR-Cas-9, TALEN, and ZFN can be used to precisely knock in genes to specific locations. These strategies may be useful for treating hereditary disorders like ALS, HD, and PD.

6.5. Other gene-related therapies

RNA aptameric therapy and epigenetic therapy are other gene-related therapies that may be used in the future.

Gene therapy has the potential to treat, prevent, or cure inherited disorders like cystic fibrosis, hemophilia, sickle cell disease, and more. However, there are still concerns about safety and clinical applications, so careful regulation and monitoring are important. (24)

6.6. Other considerations include:

6.6.1. Ethical use

It's important to maintain rigorous ethical standards as gene therapy is developed and used.

6.7. Large-scale manufacturing

Establishing a large-scale manufacturing process is required to advance gene therapy.

6.7.1. Patient selection

Frameworks for patient selection are needed to ensure the best therapeutic outcomes. (25)

7. Conclusion

Though gene therapy can offer the opportunity to significantly improve symptomology or slow progression of non-cancerous blood and/or neurologic disorders, it currently remains in the research arena. As such, those being treated do so with uncertain expectation of the outcome. It will be a number of years before a fully informed position is available, but clinicians should be thinking about who might benefit most from gene therapy based on extrapolation of early clinical trial data, potential impact on health care costs and patient quality of life.

The conclusions from these trials imply that gene therapy has the potential to cure numerous genetic diseases and that the procedures appear to have minimal risks to the patient, but the efficiency of gene transfer and the expression of the corrective genes in the human patients is still very.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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