Comprehensive Review on Gene Therapy

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Abstract :- **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. It include various type of gene therapy viz somatic and germline. Also this review include various method to enhance the drug delivery i.e chemical method and physical method to enhance drug delivery. Also in this the in vivo as well as ex vivo gene therapy were studied. Also in this Comprehensive review the various application in the various disease and various sector were studied.**

Keywords:- **Gene Technologies, Type, Methods to Enhance Delivery, Mechanism, In vivo and Ex vivo gene therapy, Application, Future aspect**

INTRODUCTION

Usually, gene therapy entails inserting a functional gene into cells to either create a new function for the cell or fix a malfunction.[1] For example, diseases such as cystic fibrosis, combined immunodeficiency syndromes, muscular dystrophy, hemophilia, and many cancers result from the presence of defective genes. The faulty genes that cause these problems can be replaced or corrected by gene therapy. Combination immunodeficiency syndromes have been particularly successfully treated with gene therapy, which has demonstrated a long-lasting and exceptional therapeutic effect.[2-4] For gene transfer, either a messenger ribonucleic acid (mRNA) or genetic material that codes for mRNA needs to be transferred into the appropriate cell and expressed at sufficient levels. In most cases, a relatively large piece of genetic material (>1 kb) is required that includes the promoter sequences that activate expression of the gene, the coding sequences that control the synthesis of proteins and signaling sequences that control the processing of RNA, including polyadenylation. Modifying a cell's endogenous gene's expression is a second type of gene therapy. This can be achieved by transferring a relatively short piece of genetic material (20 to 50bp) that is complementary to the mRNA. This transfer might impede translational beginning, mRNA processing, or even cause the mRNA to be destroyed, among other methods that could impact gene expression. On the other hand, a gene that produces complementary antisense RNA to a cellular RNA may also work similarly.

Gene therapy is a method for fixing faulty genes that lead to the onset of illness. Researchers may fix defective genes using a variety of methods, including:

• To replace a nonfunctional gene, a normal gene may be placed into an arbitrary region of the genome. This method is the most often used.

• An abnormal gene could be swapped for a normal gene through homologous recombination.

• The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.

• Gene therapy is the insertion, alteration, or removal of genes within an individual's cells and biological tissues to treat diseases. It is a technique for correcting defective genes that are responsible for disease development.[5]

Gene therapy can be broadly defined as a 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 would deliver the gene of interest into the target cells. Based on the nature of the viral genome, these gene therapy vectors could be divided into RNA and/or DNA viral vectors. The majority of RNA virusbased vectors have been derived from simple retroviruses like murine leukemia virus. A major shortcoming of these vectors is that they are not able to transduce non-dividing cells. This problem may be overcome by use of novel retroviral vectors,

derived from lentiviruses, such as human immunodeficiency virus (HIV)[6].

TYPE of GENE Therapy :-

1) Somatic gene therapy,

It involves introducing a "good" gene into targeted cells with the end result of treating the patient - but not the patient's future children because these genes do not get passed along to offspring. Stated differently, the possibility that a patient's offspring would have the same illness persists even if a portion of the patient's DNA is changed in order to treat it. Gene therapy in this way is being carried out in the majority of genetics labs worldwide.

2) Germline gene therapy

Entail injecting foreign genes into fertilized eggs or in sperms producing cells, which will then pass any genetic changes to future generations as well. But despite its potential to prevent hereditary diseases, this type of gene therapy is very contentious, and for ethical and technological reasons, not much study is being done in this field at the moment. [7]

Gene Therapy Technologies :-

The transfer of genetic material can be accomplished in vivo through local or systemic inoculation or ex vivo where the target of interest is collected and modified outside of the organism before return to the host. Transfer of synthetic DNA can be accomplished by transduction or transfection. Such methods of transfer include either direct injection of DNA into the recipient cells, or utilising methods to induce membranes permeation, receptormediated uptake or endocytosis. Recombinant viruses are used in transduction as a vehicle for gene transfer. Entry of these vectors is mediated by cell-surface receptors. Concerns regarding the immunogenicity of viral vector systems due to activation of memory responses against constituent viral proteins or a primary response to neoantigens has spawned the evolution of artificial gene delivery systems that take use of transfection, which is the physical, chemical, or electrical transfer of DNA. [8,9] Benefits of non-viral methods for DNA transfer include a reduction of risks associated with viruses (immune response, insertional mutagenesis) and limitations to gene delivery (such as length of the transgene cassette). [10]

Physical Methods to Enhance Delivery :-

1. Electroporation

Short, high-voltage pulses are used in the electroporation technique to transfer DNA across the cell membrane. It is believed that this shock creates transient holes in the cell membrane that permit DNA molecules to flow through. Electroporation is generally efficient and works across a broad range of cell types. However, electroporation's utilization, even in therapeutic settings, has been restricted due to a high cell death rate after the procedure.

2. Gene Gun

Another physical technique for transfecting DNA is the use of the gene cannon, also known as particle bombardment. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells.eg:- If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. Hematopoietic stem cells were transduced with a corrected transgene using a retrovirus in clinical trials for individuals with X-linked severe combined immunodeficiency (X-SCID), and this led to the development of T cell leukemia in 3 of 20 patients. [11]

3. Sonoporation

Sonoporation uses ultrasonic frequencies to deliver DNA into cells. It is believed that DNA can enter cells through the disruption of the cell membrane caused by the process of sonic cavitation.

4. Magnetofection

DNA is complexed to magnetic particles and brought into contact with a cell monolayer by placing a magnet beneath the tissue culture plate in a process known as magnetofection.

Chemical Methods to Enhance Delivery :-

1. Oligonucleotides

Gene therapy is the use of synthetic oligonucleotides to inactivate the genes responsible for the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Another use tiny RNA molecules known as siRNA to instruct the cell to break particular, distinctive regions in the defective gene's mRNA transcript, preventing the faulty mRNA and therefore expression of the gene.

2. Lipoplexes and polyplexes

In order to enhance the uptake of fresh DNA into the cell, the DNA has to be shielded from harm and positively charged. Originally, lipoplexes for synthetic vectors were made of neutral and anionic lipids.

3. Dendrimers

A spherical, heavily branching macromolecule is called a dendrimer. There are several methods to functionalize a particle's surface, and the surface determines a lot of the final construct's characteristics. Specifically, a cationic dendrimer can be created, that is one with a positive surface charge. When in the presence of genetic material such as DNA or RNA, charge complimentarily leads to a temporary association of the nucleic acid with the cationic dendrimer. The dendrimer-nucleic acid complex is then absorbed into the cell by endocytosis when it has arrived at its target.

4. Hybrid methods

Every gene transfer methodology has its drawbacks, which is why certain hybrid approaches that combine two or more procedures have been created. Virosomes[12] are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods[13] alone. Additional techniques include hybridizing viruses or combining different viral vectors with cationic lipids.

Electrical methods :-

Electrotransfer are more well-established. Applying an electrical field to cells alters the resting transmembrane potential, which can induce permeability though the formation of reversible structural membrane changes (electropores). A large number of animal studies have been performed across on a range of tissues, with the main application being immunotherapy.[14] Therapeutic levels of gene expression have been achieved, as well the cotransfer of multiple plasmids. [15] Although more efficient than chemical or physical methods, the efficiency of electrotransfer is still less than that seen with viral vectors. The choice between transfection strategies compared to transduction with a virus will largely depend on the therapeutic goal. Synthetic delivery technologies promise clear benefits for scenarios involving transitory gene expression or repeated dosage. Conversely, viral vectors that facilitate transgenic integration with the host may be more advantageous for the repair of missing protein diseases that call for long-term, stable gene expression. DNA and more stable constitutive protein expression. Potential advantages of synthetic delivery include ease of manufacture, low frequency of gene integration, safety, and the capacity to introduce a greater number of genes. [16,17] Another consideration is the efficacy of expression: in general, viral vectors achieve higher efficiency of expression than synthetic systems. [18,19] One potential approach to utilizing the benefits of both synthetic and viral systems is the creation of artificial viral systems, or synthetic viruses.

Mechanism of Gene Therapy :-

In order to restore the functioning of essential proteins, gene therapy modifies the genetic code. The structural foundation of the body's tissues and the workhorses of the cell are proteins. A person's genetic code contains the instructions needed to make proteins; variations, or mutations, in this code can affect the synthesis or functionality of proteins, some of which may be essential to a functioning body. Restoring or offsetting genetic alterations linked to illness may restore the function of these crucial proteins and enable normal bodily functions.

There are two ways that gene therapy can make up for genetic changes.

- Gene transfer therapy introduces new genetic material into cells. Gene transfer treatment can introduce a normal copy of the gene to restore the function of an essential protein if a changed gene renders it defective or absent. Alternatively, the therapy can introduce a different gene that provides instructions for a protein that helps the cell function normally, despite the genetic alteration.
- Genome editing is a newer technique that may potentially be used for gene therapy. I Genome editing presents gene-editing technologies that can alter the cell's existing DNA rather than introducing new genetic material. With the use of genome editing technology, specific regions of the genome can have genetic material added, deleted, or changed. CRISPR-Cas9 is a well-known type of genome editing.

Direct insertions of genetic material or gene-editing instruments into cells typically result in their non-function. Instead, a carrier called a vector is genetically engineered to carry and deliver the

material. Some viruses are utilized as vectors because they can infect cells and transfer the material. The viruses are modified so they can't cause disease when used in people. Certain virus species, including retroviruses, incorporate their genetic material—including the new gene—into a human cell's chromosome. Adenoviruses and other viruses insert their DNA into the cell's nucleus, but the DNA does not become incorporated into a chromosome. Viruses are also capable of delivering the gene-editing instruments to the cell nucleus. A single cell in a targeted bodily tissue will absorb the vector when it is injected or administered intravenously (IV). An alternative is to take a sample of the patient's cells and expose it to the vector in a lab environment. The patient is then given access to the cells harboring the vector once more. If the therapy is effective, either the editing molecules will fix a DNA mistake and restore protein function, or the new gene supplied by the vector will produce a working protein. Although using viral vectors for gene therapy has proven beneficial, there are significant risks involved. Sometimes the virus triggers a dangerous immune response. Furthermore, mistakes that result in cancer can be caused by vectors that incorporate genetic material into a chromosome. Researchers are developing newer technologies that can deliver genetic material or gene-editing tools without using viruses. One such method delivers genetic material or gene-editing components into cells using unique structures termed nanoparticles as vectors. Extremely tiny structures known as nanoparticles have been created for a variety of applications. These microscopic particles are engineered with special features to target certain cell types in gene therapy. Compared to viral vectors, nanoparticles are less likely to trigger immunological responses and are simpler to create and alter for particular uses. Researchers continue to work to overcome the many technical challenges of gene therapy. For instance, scientists are developing more effective methods for delivering genes or gene-editing instruments to specific cells. Additionally, they are trying to regulate the treatment's onset time in the body more accurately.[]

In vivo and ex vivo gene therapy :-

There are several gene delivery methods that can transmit genes in a steady or transitory manner. Non-integrating vector systems are preferred when the therapeutic impact may be attained by expressing a single gene in post-mitotic tissue. In fact, an attenuated adenovirus-derived vector was utilized to treat ornithine transcarbamylase deficiency (OTCD), an inborn urea synthesis illness, in one of the earliest in vivo clinical studies [21]. The in vivo application of vector particles was initially associated with concerns regarding vector- and transgene-elicited immunoreactions, as evidenced by the death of one of the 17 subjects treated in the OTCD trial due to a massive immune reaction against the infused adenoviral vector's capsid [22]. In the meantime, sophisticated technologies have been created, primarily in the field of cancer gene therapy, to successfully conduct clinical trials using non-integrating vectors in addition to shielding the viral capsid proteins from recognition by the host immune system [23,24]. Adeno-associated virus-derived vectors (AAV) are being utilized for the correction of monogenic diseases in post-mitotic tissues, as will be discussed in more depth later. They have demonstrated a remarkable safety profile and, when combined with additional attributes like minimal inflammatory activity, make them particularly desirable tools for in vivo gene therapy. As shown in Figure 1, Glybera® is a recombinant AAV intended for intramuscular injection. In contrast, retroviral vectors are preferred for the stable gene transfer into proliferating cells, since they have the capability to integrate into the host cell genome. The current protocols include cell isolation from the patient followed by their genetic modification outside the body and subsequent re-introduction into the patient as an autologous transplant (ex vivo gene therapy). This eliminates the possibility of germ-line transmission and reduces the potential of unintended off-target consequences, including as toxicity from the therapeutic gene's ectopic expression in off-target tissues. Furthermore, because the gene-based medication is not susceptible to metabolic or renal clearance and is less likely to elicit an immunological response, it may be delivered with greater robustness. Ex vivo gene therapy may potentially enable selection, growth, and quality control of the altered cells prior to reinfusion, depending on the technique, therefore enhancing safety and effectiveness even further (Fig 1). Due to their easy isolation from blood following G-CSF mobilization, mobilized hematopoietic stem cells (HSCs) have been used in pioneering clinical trials.

Furthermore, the substantial knowledge gained over the course of 50 years of HSC transplantation (HSCT) has benefited techniques to introduce gene-modified HSC into patients [25]. Mature blood cells have been widely employed for a wide range of gene therapy applications in tandem to HSC, leading to a broad spectrum of uses. In fact, the first time human hematopoietic cells with altered genes were used was at the

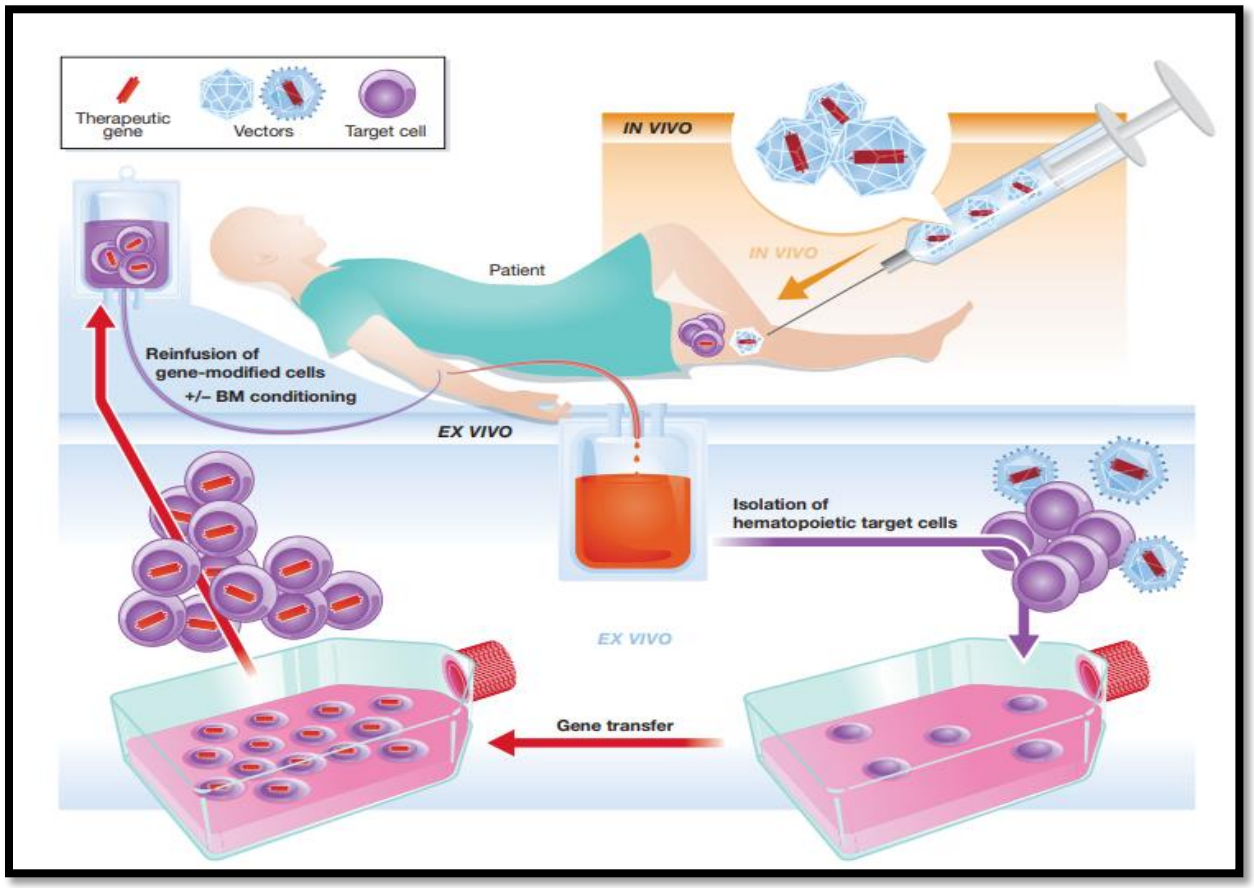

Figure 1. In vivo and ex vivo gene therapy concepts. For the in vivo application of gene-based drugs, the therapeutic gene is introduced directly into the body (e.g. muscle, liver) of the patient, while for ex vivo applications, patient cells are first isolated from the body, genetically modified outside the body and reintroduced into the patient as an autologous transplant (see text for details). BM, bone marrow.

NCI by Rosenberg et al., who inserted a bacterial gene into lymphocytes invading tumors to monitor the cells' location and durability following re-infusion into patients with metastatic melanoma [26]. After this proof of concept, T-cell genetic alteration was used to treat adenosine deaminase (ADA) deficiency in the first gene therapy study intended to repair an inherited illness [27]. Additionally, T-lymphocytes have been thoroughly tested for autologous adoptive cell transfer, which offers temporary immunotherapy lasting anywhere from a few weeks to over 10 years [28,29]. For instance, introducing an endogenous or synthetic receptor, like chimeric antigen receptors (CAR), which identify an antigen, might give T cells a new level of specificity of choice on cancer cells and thus, facilitate tumour-cell recognition, ultimately leading to formation of an armada of activated T cells and killing of target cells. In clinical studies, this strategy has been effective in treating B-cell leukemia and lymphoma, for example, by targeting the B-lymphocyte restricted surface protein CD19. This technique was well tolerated in 28 cases that were described; there were no serious adverse effects associated to the therapy, and the clinical results were hopeful, including full remissions [30,31,32]. When allogeneic hematopoietic stem cell therapy (HSCT) fails, donor-derived T lymphocytes are frequently employed to produce a graft-versus-leukemia effect.

However, the promise of this method is limited since severe graft-versus-host disease (GvHD) is often reported in treated patients, leading to lower lifetime expectancy and worsened quality of life. Patient-specific control of alloreactivity is made possible by the inclusion of inducible suicide genes into the T cell allograft, which may be triggered following GvHD development [33,34,35]. These methods will not be further examined in this review since they have already been thoroughly evaluated elsewhere [36,37,38,39,40]. As evaluated, for example, for epidermolysis bullosa, an inherited skin disorder of connective tissue, gene therapeutic approaches for skin diseases also offer a promising and readily accessible cell source for topical in vivo application [41] as well as for ex vivo modification [42]. Hepatocytes can also be extracted from the liver, cultivated ex vivo, and then genetically modified and reintroduced into the patient through the hepatic portal vein, all using very invasive techniques. In fact, Grossman et al. carried out one of the first gene therapy studies in 1992 to treat a patient with familial hypercholesterolemia by genetically modifying cultured hepatocytes ex vivo [43].

Application of Gene Therapy In Various Disease:-

1. Gene Therapy for Oral Squamous Cell Carcinoma

The current treatment strategies for oral squamous cell carcinoma (OSCC) include a combination of surgery, radiation therapy and chemotherapy. However, surgical resection of tumors frequently causes profound defects in oral functions such as speech and swallowing as well as in cosmetic aspects. [44] Chemotherapy is associated with well-known toxicity and has demonstrated no clear impact on the survival of patients with recurrent oral cancer. Recurrence develops in approximately one third of the patients despite definitive treatment. [45] Two thirds of the patients dying of this disease have no evidence of symptomatic distant metastasis. Therefore, local and regional disease control is paramount, underscoring an urgent need for more effective therapy. Several reports have indicated that the combination of radiation and gene therapies has synergistic suppressive effects on various cancer cells, including colorectal, ovarian, nasopharyngeal and head / neck cancer cells. [46] Gene therapy can also be used as an adjuvant to surgery (at the resected tumor margins). This review highlights various gene therapy methods that are available for combating OSCC.

2. Gene Therapy in Periodontics

It is believed that periodontal diseases have several etiological factors and exhibit a wide range of inflammatory and destructive responses. Variations in genes have been identified as a significant risk factor for periodontitis. With the advent of gene therapy in dentistry, significant progress has been made to control periodontal disease and reconstruct the dentoalveolar apparatus. [47] Gene therapy is a field of Biomedicine. A broad definition of gene therapy is the genetic modification of cells for therapeutic purposes. [48] Genes are specific sequences of bases present in the chromosome that form the basic unit of heredity. Each person's genetic constitution is different and the changes in the genes determine the differences between individuals. Some changes usually in a single gene, may cause serious diseases. More often, gene variants interact with the environment to predispose some individuals to various ailments. The goal of gene therapy is to transfer the DNA of interest, for example, growth factor and thrombolytic genes into cells, thereby allowing the DNA to be synthesized in these cells and its proteins (termed recombinant protein) expressed. Gene therapy may involve (1) supplying or increasing the expression of a mutant gene that is insufficiently expressed (e.g., to treat enzymatic deficiencies); (2) blocking a gene that is detrimental (e.g., using antisense constructs to inhibit tumor proliferation); or (3) adding a foreign gene to treat a situation beyond the capability of the normal genome (e.g., introduce an enzyme into a cell or tissue that allows the tissue to become more sensitive to the effects of a pharmacologic agent).

3. Gene Therapy for Cystic Fibrosis Lung Disease

The ―natural‖ approach to treating cystic fibrosis should involve gene therapy: Mutations in the CF transmembrane conductance regulator (CFTR) gene cause loss of function and are linked to the recessive illness cystic fibrosis (CF), which has a well-characterized gene product; heterozygotes, as predicted, appear to be phenotypically perfectly normal; the level of expression of CFTR in affected cells generally appears to be low; and the dysfunctional epithelial lining cells in the organ most affected by CF (the lung) are available for direct vector delivery via topical administration. [49] However, despite an impressive amount of research in this area,

there is little evidence to suggest that an effective gene-transfer approach for the treatment of CF lung disease is imminent. The difficulty to create such a therapy is partially due to the learning curve associated with vector technology and the underestimation of the ability of airway epithelial cells to protect themselves against external penetration by moieties, such as gene-therapy vectors. The problems that affect the advancement of this profession will be the main emphasis of this perspective.

4. Gene Therapy for Parkinson's Disease

Parkinson's disease (PD) is a long-term, progressive neurodegenerative condition best known for the severe movement symptoms it causes due to the extensive degeneration of dopamine nigrostriatal neurons in the midbrain. However, PD is far more complex than commonly appreciated, with multiple etiologic variables and pathogenic pathways, complex pathologies and a wide range of central nervous system (CNS) and non-CNS symptoms. The drugs' effectiveness decline with progressive pathology, leading to gradual incapacitation of patients by increased ―off‖ time (i.e., periods of no symptomatic relief) and increasing side effects such as peakdose dyskinesias. With over 4 million persons affected globally, appropriate therapy for nigrostriatal-mediated motor deficits remains a major unmet medical need. [50] Though a number of solutions have been conceived to improve the function of the degenerating dopaminergic system, translating these biopharmaceutical concepts to the clinic has been challenging due to obstacles associated with delivering macromolecules to the central nervous system in a persistent and targeted fashion. Many delivery obstacles have been addressed by advances in gene therapy (GT) during the last ten years[51], and PD is clearly a clinical indication that would benefit greatly from GT targeting many components of the disease. Major motor symptoms are associated with well-defined, localizable, and targetable neural systems; (ii) very modest titers are required. and volume of vector targeted to those sites, which avoids the systemic circulation of immunogenic materials and (iii) the large and increasing demand for improved therapeutics with an aging population, which in whole bolsters impact and financial support for research and development.

5. Gene Therapy for Infectious Diseases

Many infectious disorders that do not respond well to conventional clinical care are being studied as potential candidates for gene therapy as an alternative treatment.[52-59] Gene therapy for infectious diseases requires the introduction of genes designed to specifically block or inhibit the gene expression or function of gene products, such that the replication of the infectious agent is blocked or limited. In addition to this intracellular intervention, gene therapy may be used to intervene in the spread of the infectious agent at the extracellular level. This might be accomplished by stimulating a particular immune response or by continuously expressing an inhibitory protein released in vivo. There are three main categories into which approaches to gene therapy for viral disorders can be placed: (i) nucleic acid-based gene treatments, such as RNA decoys and antisense DNA and RNA and catalytic RNA moieties (ribozymes); (ii) protein approaches such as transdominant negative proteins (TNPs) and single-chain antibodies; and (iii) immunotherapeutic approaches involving genetic vaccines or pathogen-specific lymphocytes. It is further possible that combinations of the aforementioned approaches will be used simultaneously to inhibit multiple stages of the viral life cycle. The following important variables will directly affect how successful gene therapy will be against infectious agents: (i) choosing the right target cell or tissue for gene therapy; (ii) the efficiency of the gene delivery system; (iii) appropriate expression, regulation and stability of the gene therapy product(s); and (iv) the efficiency of the inhibition of replication by the gene inhibition product.

6. Gene Therapy for Arthritis

Rheumatoid arthritis is an autoimmune disease with intra-articular inflammation and synovial hyperplasia that results in progressive degradation of cartilage and bone, in severe cases it causes systemic complications. Recently, biological agents that suppress the activities of proinflammatory cytokines have shown efficacy as antiarthritic drugs, but require frequent administration. Thus, gene transfer approaches are being developed as an alternative approach for targeted, more efficient and sustained delivery of inhibitors of inflammatory cytokines as well as other therapeutic agents. Recently, biological agents that modulate the proinflammatory activities of TNF- and IL1 have shown efficacy as novel antiarthritic drugs. [60-63] However, arthritis therapies that employ biological agents are currently

limited by possible systemic side effects such as the occurrence and re-emergence of viral and bacterial infections as well as their exorbitant expense. There are several different approaches that can be utilized for the treatment of arthritis. [64-66] Genes can be delivered locally at the site of disease pathology such as the joint by intra-articular injection. Alternatively, therapeutic genes can be delivered using specific circulating cell types such as T cells. [67-69] or antigen-presenting cells (APCs) such as dendritic cells (DC). [70-72] Although these types of cells result in more systemic delivery of therapeutic proteins, the ability of certain immune regulatory cells to home sites of inflammation can also allow for local treatments following systemic injection. It is also possible to increase the levels of circulating therapeutic proteins by delivery of the gene to tissues such as muscle or liver. [73,74]

7. Gene Therapy in Diabetic Neuropathy

Gene therapy shows promise in treating diabetic polyneuropathy, a disorder that commonly affects diabetics who've had the disease for many years, a new study finds. Vascular endothelial growth factor (VEGF) gene injections administered intramuscularly may benefit diabetic polyneuropathy patients, according to research from Boston. The study included 39 patients who received three sets of injections of VEGF gene in one leg and 11 patients who received a placebo. Diabetic neuropathy symptoms include loss of feeling and discomfort in the legs and feet, weakness, and issues with balance. Due to the lack of feeling, foot ulcers may go unnoticed and eventually result in amputation.

Advantages of gene therapy

- In case of ‘silence’ a gene. By employing gene therapy to "silence" the virus before it manifests, doctors might spare an individual with HIV who has not yet progressed to AIDS the agony and suffering caused by the illness.
- Gene therapy has the potential to eliminate and prevent hereditary 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.[5]

Future aspects of gene therapy :-

- Nanotechnology + gene therapy yields treatment to torpedo cancer. March, 2009. The School of Pharmacy in London is testing a treatment in mice, which delivers genes wrapped in nanoparticles to cancer cells to target and destroy hard-toreach cancer cells. Read BBC article.
- Results of world's first gene therapy for inherited blindness show sight improvement. 28 April 2008. The first-ever clinical trial findings have been released by UK researchers from the UCL Institute of Ophthalmology and Moorfields Eye Hospital NIHR Biomedical Research Centre. The experiment was designed to assess a novel gene therapy treatment for a particular kind of hereditary blindness.
- Researchers at the National Cancer Institute (NCI), part of the National Institutes of Health, successfully reengineer immune cells, called lymphocytes, to target and attack cancer cells in patients with advanced metastatic melanoma. This is the first time that gene therapy is used to successfully treat cancer in humans. See New Method of Gene Therapy Alters Immune Cells for Treatment of Advanced Melanoma (August 30, 2006).
- Gene therapy is effectively used to treat two adult patients for a disease affecting non lymphocytic white blood cells called myeloid cells. Numerous bone marrow failure syndromes, including acute myeloid leukemia, are examples of the common myeloid diseases. This is the first research demonstrating the potential of gene therapy to treat myeloid system disorders. See Gene Therapy Appears to Cure Myeloid Blood Diseases In Groundbreaking International Study (March 31, 2006)..
- University of California, Los Angeles, research team gets genes into the brain using liposomes coated in a polymer call polyethylene glycol (PEG). A major accomplishment is the transfer of genes into the brain, as viral vectors are too large to pass across the "blood-brain barrier." Parkinson's disease may be treated using this approach. See March 20, 2003, article "Undercover Genes Slip into the Brain."
- RNA interference or gene silencing may be a new way to treat Huntington's. Short pieces of double-

stranded RNA (short, interfering RNAs or si RNAs) are used by cells to degrade RNA of a particular sequence. If a si RNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced. See Gene Therapy May Switch off Huntington's (March 13, 2003).

- New gene therapy approach repairs errors in messenger RNA derived from defective genes. Technique has potential to treat the blood disorder thalassaemia, cystic fibrosis, and some cancers. See Subtle Gene Therapy Tackles Blood Disorder (October 11, 2002).
- Researchers at Case Western Reserve University and Copernicus Therapeutics are able to create tiny liposomes 25 nanometers across that can carry therapeutic DNA through pores in the nuclear membrane.Sickle cell is successfully treated in mice.[5]

CONCLUSION

According to gene therapy different types of genetic disorder are cured. In case of cystic fibrosis, Diabetes, AIDS, Hepatitis melanoma, Alizhmer, Parkinson's diseses etc. As gene therapy is uprising in the field of medicine, scientists believe that after 20 years, this will be the last cure of every genetic disease. Genes may ultimately be used as medicine and given as simple intravenous injection of gene transfer vehicle that will seek our target cells for stable, site-specific chromosomal integration and subsequent gene expression. Gene therapy is on its way to fulfill the early promises made just two decades ago despite the occurrence of severe side effects observed in the initial clinical trials. The risks associated with gene therapy are already being successfully addressed or are the focus of current research as presented here.

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