ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Gene therapy in current medical arena

Arun Chander Yadav. K, Jayasudha. D, Anusha. N

Manuscript Info

Manuscript History:

Received: 14 October 2015
Final Accepted: 25 November 2015
Published Online: December 2015

Key words: Gene therapy, alipogenetiparvovec, aav vector, lipoplexes

Corresponding Author

Arun Chander Yadav. K
arunkrishnamurthy89@gmail.com

Abstract

Gene therapy is an experimental technique that uses genes encoding the expression of proteins that are either endogenous or biological to treat or prevent a disease by inserting it into patient's cells. Previously the challenges faced were the gene titers which then changed to their delivery and now expression of the genes. Initially the trial of gene therapy for adenosine deaminase deficiency had proven to be successful but till date only alipogenetiparvovec has been approved in Europe for lipoprotein lipase deficiency. Recent techniques with adenovirus, r-adeno associated virus (r-aav), retrovirus,herpes simplex virus vectors and non-viral lipoplexes (for transfer of large biological products) have completely revolutionized the future of gene therapy. With the upcoming advances in the field of cancer therapy such as herpes simplex thymidine kinase (hs-tk) and its role in suicidal gene therapy in the treatment of cancers has stretched new horizons in the field of gene therapy. Recently the newer treatment approaches with RNA interference and anti-sense oligonucleotides in muscular dystrophies were certainly encouraging for the researchers to further work on the newer prospects of gene therapy. Gene therapy is currently investigated for cardiac failure and for prevention of brain ageing in Alzheimer's disease.

INTRODUCTION

Gene therapy is an experimental technique that uses genes to treat or prevent a disease by inserting a gene into a patient's cells instead of using drugs or surgery. Researchers are testing several approaches to gene therapy including:

- ✓ Replacing a mutated gene that causes disease with a healthy copy of the gene.
- ✓ Inactivating, or "knocking out," a mutated gene that is functioning improperly
- ✓ Introducing a new gene into the body to combat a disease.

Sickle-cell anemia, a disease affecting red blood cells, was the first genetic disorder discovered in the history of gene therapy. The first gene therapy trial conducted in 1991, which was designed to treat an immune system disorder known as adenosine deaminase(ADA) deficiency created the benchmark in the history of gene therapy. There were only two patients in that trial, one of whom showed a modest recovery, while the second patient, a young girl named AshiDeSilva, showed a dramatic improvement. This trial proved to the research community that gene therapy could work. Indeed, a trial conducted at the University of Pennsylvania in 1998 ended in disaster when one of the patients, a young man named Jesse Gelsinger, died as a direct result of the treatment. The consequences of this trial were profound as they affected not only gene therapy but also all experimental therapies that involve human subjects.(1) However in the past two decades, endless efforts of researchers in various fields of gene therapy such as development of efficient gene delivery vector systems, discovery of various new gene mutations in diseases and newer concepts in pathophysiology of disease manifestations all together has put gene therapy back into the field of medicine.

Types of gene therapy:

- Germ line: Germ cells (sperm or egg) are modified by the introduction of functional genes, which are integrated into their genome. At present it is banned.
- Somatic: Therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only and are not heritable with few exceptions.(1)

Techniques:

- **Vectors:**

All gene therapy approaches use a vector to deliver the genetic material to cells and also utilize the transcriptional machinery of the cell for gene transcription. The first gene therapy success of X-linked Severe Combined Immunodeficiency (SCID) patients was using a retrovirus as a vector.

- Types:
  1. Viral vectors
  2. Non-viral vector

Viral vectors:

Viral vectors consist of a genetic material surrounded by a protein-based capsid or a lipid envelope that interacts with specific cell surface receptors to aid binding, internalization, and delivery of the therapeutic gene into the target cell.(2)

Adenovirus:

- Advantages:
  - Can carry about 30 kb DNA
  - High functional titers
  - Capability of transducing dividing as well as non-dividing cells
  - Fast transgene expression (1-2 days post-delivery)
- Disadvantages:
  - Short duration of expression
  - Immunogenicity.(2)

- **Retrovirus**:

Retroviral vectors are created by removal of the retroviral gag, pol, and env genes. These are replaced by the therapeutic gene.

Advantages:

- Ability to transform its single stranded RNA to double stranded DNA
- Can be used to permanently modify the host cell nuclear genome.

Disadvantages : Even though they are used in most of the experiments as a vector most of the studies have revealed the problem of insertion mutagenesis due to the expression of the enzyme integrase.(3)

Adeno associated virus :

Adeno-associated virus (aav) vectors are the most favourable viral delivery method for long-term transgene expression.

- Advantages:

They are non-pathogenic in humans with low risk of insertion mutagenesis and moreover it depends on helper virus for their active replication, without its helper virus its genome remains in a latent state because its integrative capacity was eliminated by removal of rep and cap at their inverted terminal repeats(itr's) region.After the helper virus co-infection/co-transfection, aav starts replicating and will release the desired therapeutic gene products.

- Disadvantages:

Short term expression and can carry only small amount of genome. (4)

❖ Herpes simplex virus as vector:

-They exist in 2 forms: replication defective; replication competent.

- Advantages:
  - Persistence of gene expression
  - herpessimplex thymidine kinase (hs-tk) sensitizes the tumor cells to ganciclovir which is a natural substrate for hs-tk. Thus the enzymatic process induced by GCV leads to death of a natural substrate for hs-tk.
  - Capable of inserting large DNA sequences.

- Disadvantages:
  - inadequate penetration into the target interstitial space
  - significant uptake of hsv in normal organ
  - immunogenicity
  - insertion mutagenesis (5)

Non-viral vectors

❖ Initially direct injection of naked plasmid DNA near or into the site of injury has been the method of choice in gene therapy using non-viral techniques.

❖ Recent techniques involves:
  - Lipoplex-mediated gene delivery (lipids+ liposomes interact with plasmids to form lipoplexes) action by Sonoporation.
  - Polyplex-mediated gene delivery (complexes of cationic polymers condensing –ve charged DNA by ionic interactions) action by proton sponge effect.
  - Dendrimer-mediated gene delivery (as promising carriers in siRNA delivery)
  - Graphene-mediated gene delivery (Polyethylenimine(PEI) + Graphene oxide + DNA complexes= effectively deliver plasmid DNA into cells)

- Advantages:
  - Easily manufactured and produced in large numbers using genetic engineering techniques
  - No risk of neutralizing antibodies interference in therapy.
  - Can carry large amount of DNA.
  - Currently used in significant number of gene therapy trials.

- Disadvantages:
  - Short term gene expression.
  - Relatively low transfection efficiency compared to viral vectors.
  - Rarely immunogenic reactions.

❖ Nano-particle technology using antisense oligonucleotides(ASO) for RNA targeting therapies:
  - One of the most promising approaches to modulate mi-RNA expression is the development of single-stranded ASOs that directly bind to the target mi-RNA to inhibit their function and thus causing translational repression of the target gene.

- ASO's can also modulate pre-mRNA splicing occurring because of mutations.

Fomivirsen for CMV retinitis and Mipomersen for familial hypercholesterolemia are till date approved ASO's. (6)

CLINICAL TRIALS CURRENT SCENARIO

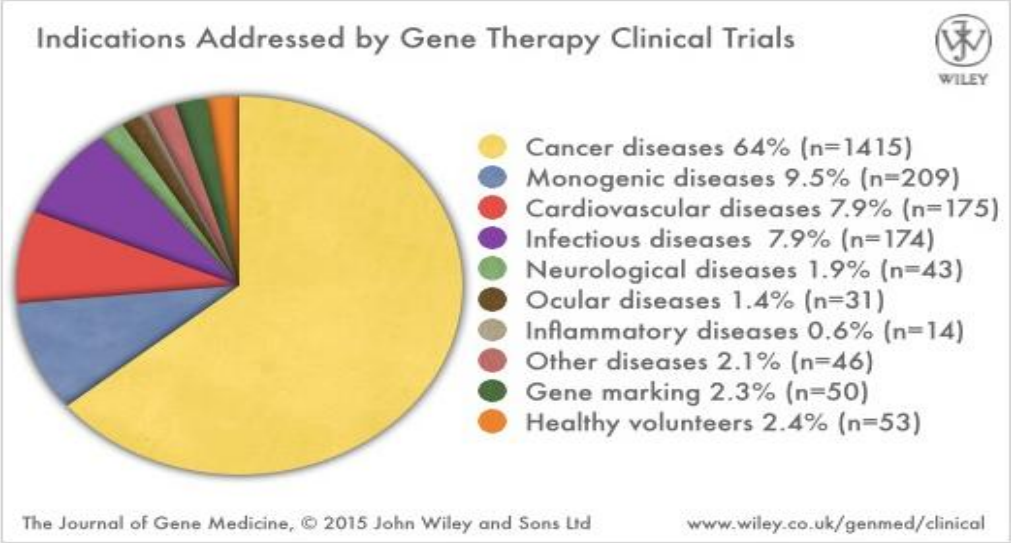

Figure 8: Indications addressed by gene therapy trials

Source: http://www.wiley.com/legacy/wileychi/genmed/clinical/

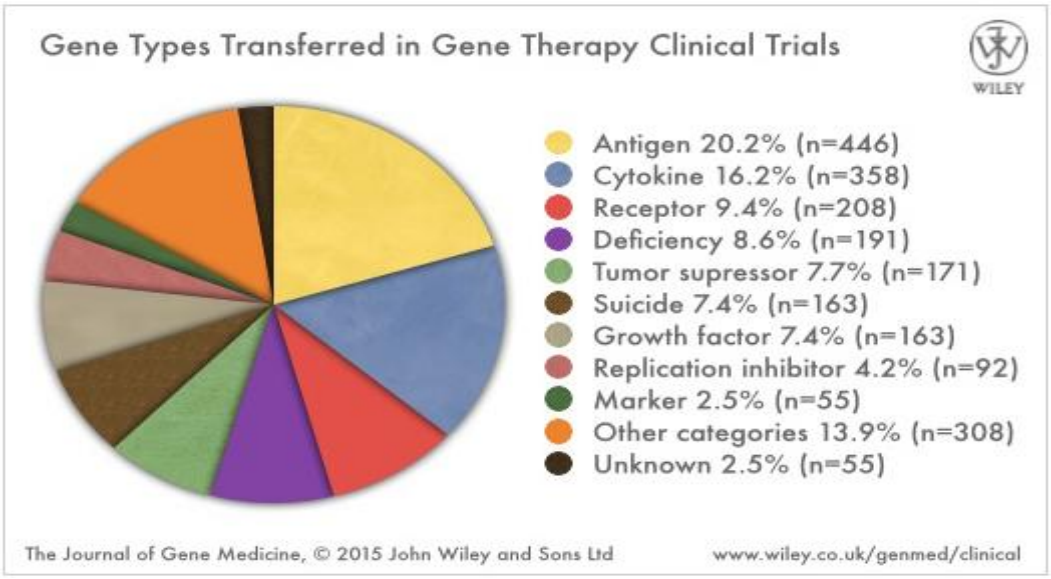

Figure 9: Gene types transferred in gene therapy clinical trials

Source: http://www.wiley.com/legacy/wileychi/genmed/clinical/

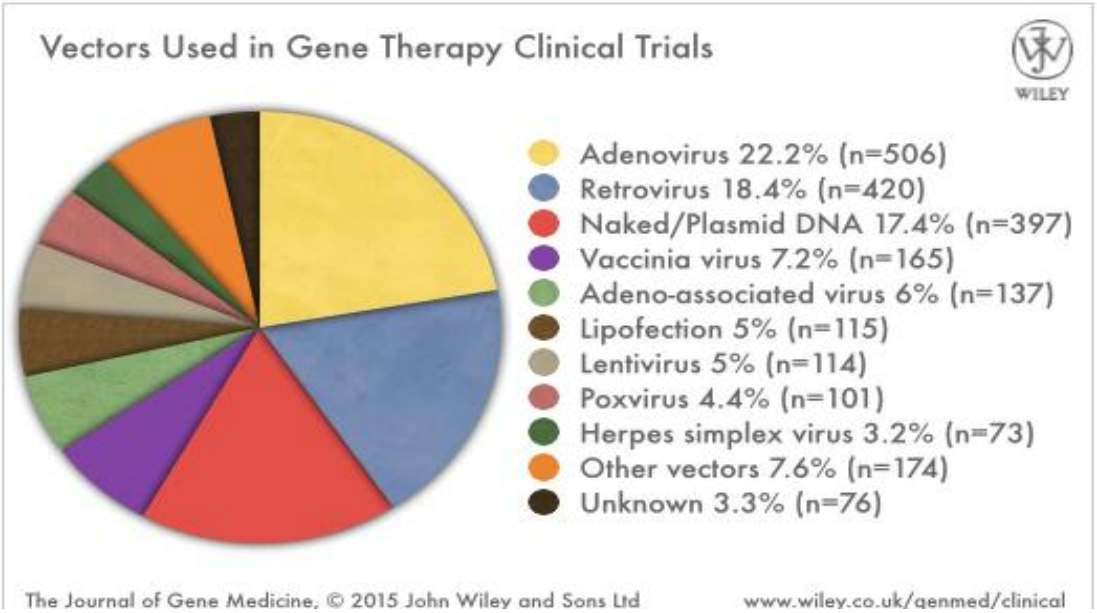

Figure 10: Vectors used in gene therapy clinical trials

Source: http://www.wiley.com/legacy/wileychi/genmed/clinical/

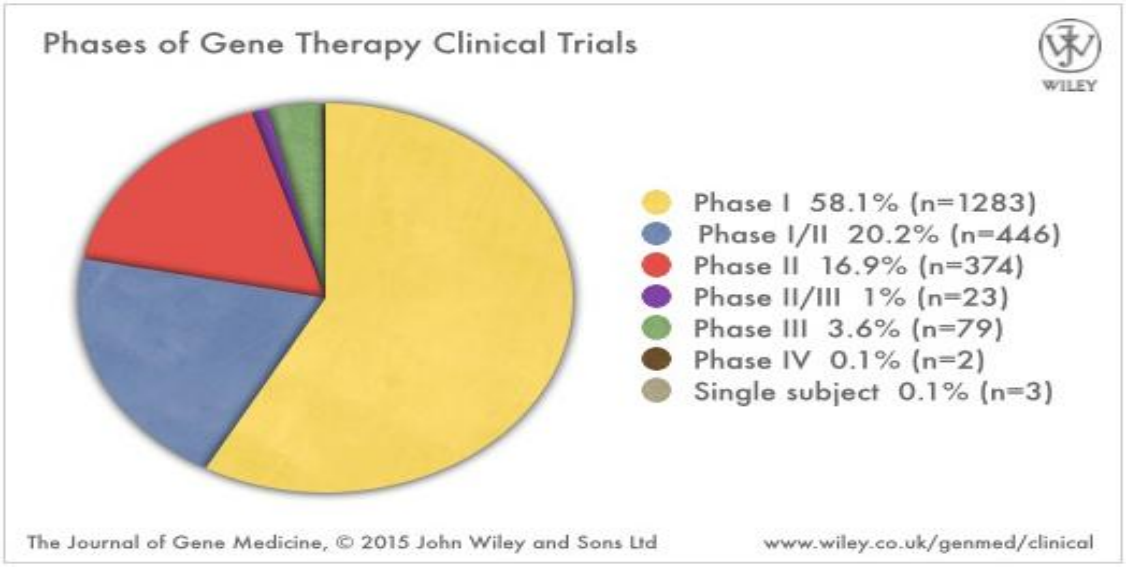

Figure 11: Phases of gene therapy clinical trials

Source: http://www.wiley.com/legacy/wileychi/genmed/clinical/

- **Areas of focus**:
  - Cancer (colon cancer, brain tumors, melanoma, esophageal cancer)
  - Hemophilia-B, SCID, cystic fibrosis, Duchenne'smuscular dystrophy
  - Cardiovascular diseases
  - AIDS
  - Alzheimer's disease

❖ **CANCERS**

- **Colon cancer**:

In colon cancer MSH-2 on chromosome 2 & MLH-1 on chromosome 3 code for proteins involved in post replicative mismatch repair of DNA.So, when mutation occurs in these genes it may result in lack of repair enzyme. There is also a gene called survivinwhich is over expressed in colon cancer and involved in chemo-resistance, angiogenesis and poor prognosis. In studies recombinant aav mediated survivin mutant thr34ala was found to inhibit the cell proliferation, induce apoptosis and mitotic catastrophe, and sensitize the colon cancer cells to chemotherapeutic drugs in vitro. It also has strongly enhanced the anti-tumor activity of oxaliplatin in animal studies.Thus aav mediated gene transfer in combination with chemotherapy may be a promising approach in treating colon cancer. (7)

- **Brain tumors**:

They are highly vascular tumors which at present facing many challenges in chemotherapy. Management of this tumor includes selective targeted delivery of chemotherapeutic agents and inhibiting the angiogenesis. Cyototoxic/suicidal gene therapy with herpes simplex thymidine kinase (hs-tk) sensitizes the tumor cells to ganciclovir which is a natural substrate for hs-tk. Thus the enzymatic process induced by GCV leads to death of a natural substrate for hs-tk(i.e. tumor cells). Also a numberof anti-angiogenic factors have been expressed from herpes simplex virus. One of which is G47 which is combined with angiostatin (endogenous inhibitor of angiogenesis). Promising results have been shown in mice i.e. decreased micro vascular density & VEGF expression.Thus anti-angiogenesis in combination with suicide gene therapy could be a novel approach in the treatment of brain tumours.(8)

- **Melanoma**:

  In melanoma there is a point mutation in CDKN2 on chromosome 9, this CDKN2 codes for a protein called p16 which when defective leads to uncontrollable cell division.Chemotherapy and surgery plays an important role in the management of melanoma but the recurrence is common with these measures.Suicidal gene therapy using hsv-tk is in the phase 1 clinical trials. (9)

Esophageal Cancer:

It is an aggressive tumor with poor prognosis. At the molecular level there is an over expression of miR-34a which down regulates apoptosis and modulates the cell division.Thus by the process of silencing the miR-34a can result in regression of tumor. Another combination therapy tried in locally advanced resectable esophageal cancer is combination therapy viz, Tumor Necrosis Factor(TNF)+radiation+5 –Fluro Uracil+ cisplatin. TNF is a replication deficient (E1,E3 and E4 deleted) adenovirus containing the gene for TNF-alpha controlled by a radiation inducible promoter. This allows the expression of TNF-alpha to be the greatest in the area receiving radiation.TNF-alpha is a potent cytokine that has been shown to have potent anti-cancer activities but could not be delivered at effective doses due to systemic toxicity. Hence the combination therapy could pave the selective delivery of TNF alpha to tumor cells. (10)

❖ **Hemophilia B**:

A X-linked recessive disorderthat leads to factor IX deficiency in which females are the carriers and males are affected. With the help of gene therapy that is mediated by adeno associated virus (aav8) has shown to raise factor IX levels for upto 16 months. Also with a follow up period of 3 years, no late toxic effects from the therapy were reported. Noted adverse event was increase in ALT levelswhich subsided after prednisolone therapy. (11)

❖ **Severe Combined Immunodeficiency(SCID-X1)**

In SCID-X1 a mutation occurs in IL2R gene which is a critical component of IL-2, IL-4, IL-7, IL-9, IL-15, IL-21. SCID-X1 is characterized by the complete lack of T cells and natural killer cells whereas B cells are present. In a study done in France by Hacein-Bey-Abina S, *et al*. with 9 patients who all lacked an HLA-identical donor wereincluded in the study. They underwent *ex vivo* retrovirus –mediated transfer of gamma chain to autologous CD34 cells. After nearly 10 years of follow up, gene therapy was shown to have corrected the immunodeficiency associated with SCID-X1. Out of 9 patients, 4 of them developed acute leukemia. (12)

- **Cystic Fibrosis**:
  Cystic fibrosis is caused by a mutation in a gene that code for a sodium chloride transporter called CFTR which is found on the surface of the epithelial cells that line the lungs and other organs.Cationic lipid-mediated CFTR gene transfer to the patients through nebulization resulted in significant correction of chloride abnormality in the patients receiving treatment compared to placebo. Bacterial adherence was also reduced. (13)

- **Duchenne's Muscular Dystrophy(DMD):**
  DMD is caused by loss of function mutations in the X-linked DMD gene (dystrophin) resulting in near complete deficiency of dystrophin in cardiac, skeletal and smooth muscles. Expression of even 20-30% of dystrophin levels is sufficient to avoid muscular dystrophy in mice and humans. Premature termination codon(PTC) mutations are found in dystrophin gene and this knowledge led to the discovery of Ataluren, which is can correct this PTC mutation was effective in restoring functional dystrophin expression in mice. As of march 2013, a phase 3 study of ataluren was initiated which restricted to the DMD patients with PTC mutations. Antisense oligonucleotides also were useful in restoring the dysrophin expression by the method of exon splicing. Two ASO's which are in current clinical trials are Drisapersen and eteplirsen. (14)

- **Cardiovascular diseases**:
  Human left ventricle has 2-4 billion cardiomyocytes. Disorders of cardiac overload such as hypertension or valvular heart disease and associated with ageing results in loss of around 20 million cardiomyocytes per year. It is estimated that 25% of cardiomyocytes wiped out in a few hours of myocardial infarction. The molecular target for cardiovasculardiseaseswhich are introduced through its respective vectors are as follows:-**VascularEndothelialGrowth Factor-A** using aav1 and aav-6 has shown improvement in tissue viability and cardiac function along with reduced apoptosis in animal studies; **Fibroblast Growth Factor 4** using adenovirus 5 in a stress induced myocardial ischaemia in animal studies have shown to improve regional myocardial function and perfusion after 12 weeks of post injection; **Sarcoplasmic reticulum ca^{2+}-ATPase** using adenovirus 5 done in human ventricular myocytes from patients with end-stage heart failure have shown increased pump activity and contraction velocity and also improved calcium concentrations in systole and diastole; **S100A1** is preferentially expressed in myocardial tissue and it exerts profound inotropic actions through the modulation of cardiomyocyte calcium homeostasis and myocardial filament function independent of beta adrenergic stimulation. It has been shown that Cardiomyocytes that over express S100A1 seems to have a higher content of ATP in them.S100A1 using aav9 have shown restoration of S100A levels with improved calcium handling, energy homeostasis and improvement in cardiac contractility in animal studies.(15)

- **Acquired Immunodeficiency Syndrome(AIDS**):
  RNA interference is a newer concept in the treatment of HIV-1 infection which involves the pairing of short mi-RNAs to an endogenous mRNA target, which leads to silencing of gene, thus impeding the further transcription of mRNA thereby preventing the active viral replication. These are done both as ex vivo using a lenti virus vector and in vivo using adeno associated virus as vector. Since these studies are still in pre-clinical stages detailed exploration of these concepts are to done in future. (16)

- **Alzheimer's disease:**
  It is a polygenic disease with defects in AD gene. Of which AD3 gene located on chromosome 14 and AD4 gene located on chromosome 1 code for amyloids which on mutation will make them turn out to be the potent neurotoxins. In the recent years many newer studies revealed the role of calcium and its effect on brain ageing. Brain calcium regulatory processes are altered during ageing disrupting neuronal and cognitive functions. In hippocampal pyramidal neurons, the calcium dependent slow after hyperpolarization (sAHP) shows an increase with ageing is because of the ryanodine mediated calcium release and elevated L-type calcium channel activity. Recent studies have found out that FK-506 binding protein (FKBP1b), a small protein that regulates calcium, declines with ageing in the hippocampus. Micro injections of adenoassociated virus bearing a transgene encoding FKBP1b when given into the hippocampus of aged rats confirmed hippocampal FKB1b overexpression 4-6 weeks after the injections. Compared to aged vector controls,aged rats overexpressing FKB1b showed dramatic enhancement of spatial memory, which correlated with marked reduction of sAHP magnitude. Thus hippocampal FKB1b overexpression reversed key aspects of calcium dysregulation and cognitive impairment in aging rats, supporting the novel hypothesis that declining FKB1b is a molecular mechanism underlying ageing related calcium dysregulation and pointing to FKB1b as a potential therapeutic target. (17)

- **Regulatory Bodies:**

I. **NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES:**

II. The purpose of the NIH Guidelines is to specify the practices for constructing and handling: (i) recombinant nucleic acid molecules, (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (iii) cells, organisms, and viruses containing such molecules. The "**Office of Biotechnology Activities (OBA)**" is the office within the NIH that is responsible for reviewing and coordinating all activities relating to the NIH Guidelines. "**Recombinant DNA Advisory Committee**" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant or synthetic nucleic acid molecule research. The NIH Guidelines are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. (18)

III. **Center for Biologics Evaluation and Research (FDA - CBER):**

IV. FDA-CBER is involved in authorizing the Investigational New Drug (IND) application. Collaborating with Principal Investigators, Institutional Biosafety Committees, Institutional Review Boards FDA ensures the safety and regulates the compliance of human gene transfer procedures. (18)

Other major regulatory institutes are as follows:

V. **European Union – Biotechnology**

VI. **The European Medicines Agency (EMA)**

VII. **Gene Therapy Discussion Group of The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)**(18)

- **Gene therapy product approval by FDA and EU:**
- The first gene therapy approved for clinical by the Chinese state Food and Drug Administration in October 2003 was **Gendicine**, an adenovirus vector carrying the p53 gene. It was developed by SibionoGenetech co. of Schenzen, china, but this approval was made on the basis of tumor shrinkage rather than extension of patient lifetime. (19)
- The European Union in November 2012 approved for the first time a gene therapy product Glybera which contains **alipogenetiparvovec** as the active substance. The drug works by breaking down the chylomicron particles present in the blood. It delivers a normal LPL gene into the body for correcting the LPL deficiency. The drug normalizes the metabolism of fat in the blood and thereby prevents episodes of pancreatitis. The drug is administered in the form of an injection into the leg muscle. It was developed by Uniqure, a Dutch biotech company.(20)

- **Safety Issues:**

Genetic Modification Clinical Research Information System(GEMCRIS):

The Genetic Modification Clinical Research Information System (GeMCRIS) is a web-based information system for human gene transfer trials that was developed in collaboration with the Food and Drug Administration (FDA) and is designed to facilitate safety reporting in collaboration with Office of Biotechnology Analysis (OBA) under NIH guidelines. Investigator and sponsors of a human gene transfer trials can utilize this system to report serious adverse events (SAEs) and annual reports. A hard copy of the electronic report can be printed and used to fulfill FDA reporting requirements. GeMCRIS also allows members of the public to access the basic reports about human gene transfer trials registered with the NIH and to search for information such as trial location, the names of investigators conducting trials, and the names of gene transfer products being studied. (21)

- **Limitations of Gene Therapy:**
  - Viral carriers even though they are more efficient compared to other vectors in gene therapy, they are typically costly, difficult to produce in large quantities.
  - Use of viral vectors which leads to the immunogenic reactions.
  - Short term expression in vectors carrying the desired genes.
  - Development of T-cell leukemia in Patients in follow up studies.
  - Lack of tissue selectivity by the vectors.
  - Intracellular degradation of gene carriers.
  - In some cases there may be pre-existing neutralizing antibodies of viral carriers in the circulation.

- Exploration of further knowledge about the pathophysiology of diseases.
- Even some pathophysiological mechanisms which are discovered have proven to be effective only in animal studies.
- Human clinical trials being tried were under progress and in the early phase of trials.
- Very few trials which reached to phase 3 clinical trials have a great task ahead to get the FDA approval.

❖ **Future challenges:**

- Development of viral vectors in large quantities with cost effectiveness, long term expression with tissue specificity and less immunogenic and non-mutagenic properties.
- Development of gutless adenoviral vectors to reduce immunogenicity.
- Inventing newer technologies to carry the non-viral vectors with desired genes into the patient's cell preventing them from intracellular enzyme degradation.
- Glybera (alipogenetiparvovec) is now the first gene therapy product approved by the European Union (EU) is being the future challenges for other researchers in the field of gene therapy to work extensively to bring out many novel agents into the field of gene therapy.
- With the identification of FKB1b target in brain ageing in pre-clinical studies, future targets would be exploring more about this gene expression in clinical studies.
- Many more advancements in newer techniques such as RNA interference and ASO's in future may further progress to the levels of FDA approval for its use in many indications.

❖ **Summary:**

Over the last two decades gene therapy is moving in a steady phase from the success of gene therapy in SCID to the latest European Union approval of gene therapy product alipogenetiparvovec. Various newer delivery techniques were developed such as a novel adeno associated viral vectors and several non-viral vectors such as lipoplexes to till date Graphene Oxide (GO) mediated gene therapy. Several pre-clinical to clinical diseases in the advancements of Cardio vascular disease researches such as molecular target involving VEGF-A, FGF 4,Sarcoplasmic reticulum calcium ATPase and more recently S100A1 using viral vectors has led to the greater improvements in the field of gene therapy. In pre-clinical studies of cancers following advancements in gene therapy are as follows

(i) In colon cancer aav mediated survivin mutant thr34ala have shown to sensitize the tumor cells to oxaliplatin.

(ii) In brain tumors herpes simplex thymidinekinase (hs-tk) acts as a natural substrate to ganciclovir and sensitize the tumor cells to ganciclovir.

(iii) Suicidal gene therapy using hs-tk in melanoma and TNF in combination with chemotherapy and radiotherapy using adenovirus as a vector shown to reduce the tumor size in animal

Thus all these studies paved the positive way for the gene therapy in cancer trials. Nevertheless further improvements in newer methods such as RNA interference approaches in HIV adding to this with the introduction of cationic liposomes mediated CFTR gene transfer in cystic fibrosis and Anti-Sense Oligonucleotides(ASO) in the treatment of muscular dystrophies will lead to further advancements in gene therapy approaches. Recently, studies have shown that declining FKB1b is a molecular mechanism underlying ageing -related calcium dysregulation and pointing to it FKB1b overexpression could be a potential therapeutic target in Alzheimer's disease.

❖ **Conclusion**:

As our understanding of disease pathophysiology and newer techniques in vector development have improved significantly from the past decades many novel targets for gene therapy could be identified in near future. Recent advances in pharmacogenomics will allow us to choose the specified patients for gene therapy. To address the challenges faced in the field of gene therapy Clinicians, Researchers and Genetic engineers should all join to produce greater efforts in the field of gene therapy. Nevertheless gene therapy could also turn out to be a serious burden of the patients experiencing untoward effects of it. Hence utmost care and attention in the pre-clinical and clinical studies must be ensured and the proper safety and ethical guidelines to be followed by the researchers to make the future of gene therapy as a promising one.

References:

1. Panno J. Gene therapy: treating disease by repairing genes. New York: Facts on File; 2005.12-30.
2. Gould D. Gene doping: gene delivery for olympic victory. Br J ClinPharmacol. 2013;76:292–8.
3. Yi Y, Noh MJ, Lee KH. Current advances in retroviral gene therapy. Curr Gene Ther. 2011;11:218–28.
4. Chtarto A, Bockstael O, Tshibangu T, Dewitte O, Levivier M, Tenenbaum L. A next step in adeno-associated virus-mediated gene therapy for neurological diseases: regulation and targeting.Br J ClinPharmacol. 2013;76:217–32.
5. Manservigi R, Argnani R, Marconi P. HSV Recombinant Vectors for Gene Therapy. Open Virol J.2010;4:123–56.
6. Ewert KK, Zidovska A, Ahmad A, Bouxsein NF, Evans HM, McAllister CS, *et al.* Cationic Lipid–Nucleic Acid Complexes for Gene Delivery and Silencing: Pathways and Mechanisms for Plasmid DNA and siRNA. Top Curr Chem. 2010;296:191–226.
7. Mobahat M, Narendran A, Riabowol K. Survivin as a Preferential Target for Cancer Therapy. Int J Mol Sci. 2014;15:2494–516.
8. Murphy AM, Rabkin SD. Current status of gene therapy for brain tumors. Transl Res J Lab Clin Med. 2013;161:339–54.
9. Zarogoulidis P, Darwiche K, Sakkas A, Yarmus L, Huang H, Li Q, *et al*. Suicide Gene Therapy for Cancer Current Strategies. J Genet Syndr Gene Ther . 2013;45:145-55.
10.Gene therapy net [Internet].[cited 2015 Aug 21]; Available from: http://www.genetherapynet.com/clinicaltrialsgov.html
11. Nathwani AC, Reiss UM, Tuddenham EGD, Rosales C, Chowdary P, McIntosh J, *et al*. Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B. N Engl J Med. 2014 ;371:1994–2004.
12. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, *et al*. Efficacy of Gene Therapy for X-Linked Severe Combined Immunodeficiency. N Engl J Med. 2010 Jul 22;363:355–64.
13. Manunta MDI, McAnulty RJ, Tagalakis AD, Bottoms SE, Campbell F, Hailes HC, et al. Nebulisation of Receptor-Targeted Nanocomplexes for Gene Delivery to the Airway Epithelium. PLoS ONE [Internet]. 2011;6:275-85.
14. Seto JT, Bengtsson NE, Chamberlain JS.Therapy of Genetic Disorders—Novel Therapies for Duchenne Muscular Dystrophy.CurrPediatr Rep. 2014;2:102–12.
15. Mason D, Chen Y, Krishnan HV, Sant S. Cardiac gene therapy: Recent advances and future directions. The Journal of controlled release.2015; 71:215-35.
16. Méndez-Ortega MC, Restrepo S, Rodríguez-R LM, Pérez I, Mendoza JC, Martínez AP, *et al*. An RNAi in silico approach to find an optimal shRNA cocktail against HIV-1.Virol J. 2010;7:369-80.
17. Gant JC, Chen KC, Kadish I, Blalock EM, Thibault O, Porter NM, *et al*. Reversal of Aging-Related Neuronal Calcium dysregulation and Cognitive Impairment by Delivery of a Transgene Encoding FK506-BindingProtein 12.6/1b to the hippocampus. The Journal of Neuroscience. 2015; 35:10878-87
18.Office of Biotechnology Activities [Internet]. [cited 2015 Aug 21]; Available from: oba.od.nih.gov/rdna_rac/rac_about.html
19. Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2007-an update. J Gene Med. 2007;9:833-42.
20.First Gene Therapy Recommended For European Approval [Internet]. [cited 2015 Aug 21]; Available from: http://www.medicalnewstoday.com/articles/248102.php
21.Office of Biotechnology Activities [Internet]. [cited 2015 Aug 21]; Available from: http://oba.od.nih.gov/rdna/oba_gemcris_pi.html