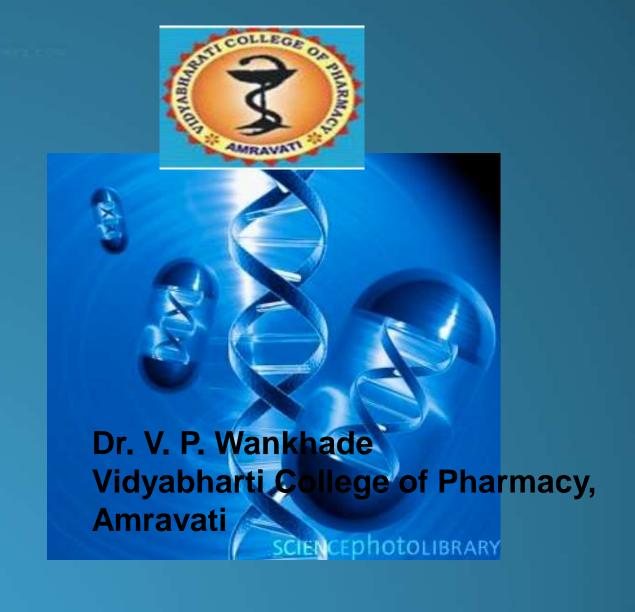
Immobilization of Enzymes



INTRODUCTION

- The term "immobilized enzymes" refers to "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously."
- Enzymes have the ability to catalyze reactions under very mild conditions with a very high degree of substrate specificity, thus decreasing the formation of by-products. Among the reactions catalyzed are a number of very complex chemical transformations between biological macromolecules, which are not accessible to ordinary methods of organic chemistry. This makes them very interesting for biotechnological use.

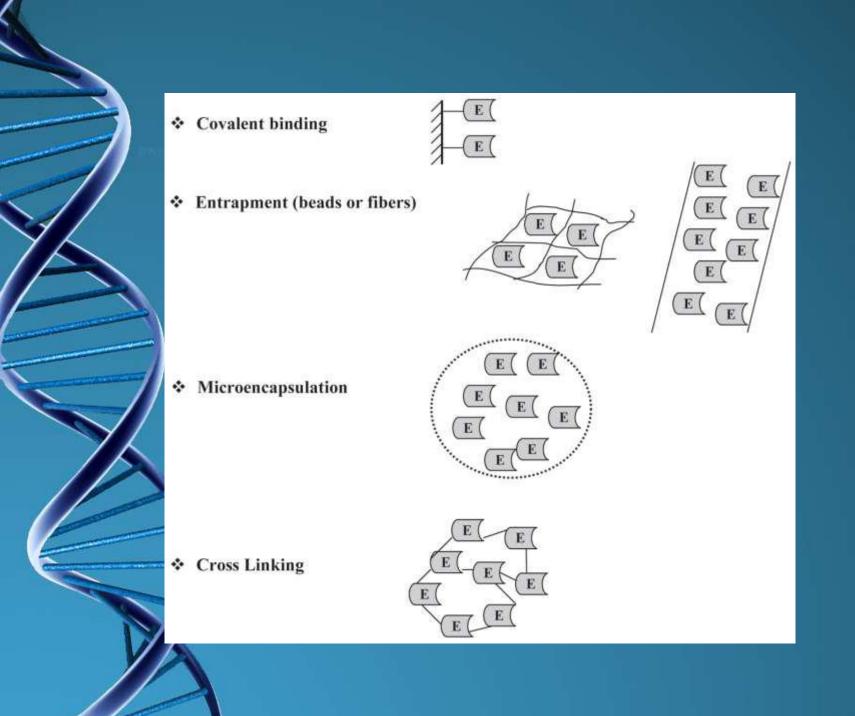
- Classification of Supports
- Organic
 - /Natural polymers
- Polysaccharides: cellulose, dextrans, agar, agarose, chitin, alginate
- Proteins: collagen, albumin
- Carbon
- Synthetic polymers
- Polystyrene
 - Other polymers: polyacrylate polymethacrylates, polyacrylamide, polyamides,
 - vinyl, and allyl-polymers
 - Inorganic
- Natural minerals: bentonite, silica
- Processed materials: glass (nonporous and controlled pore), metals, controlled pore

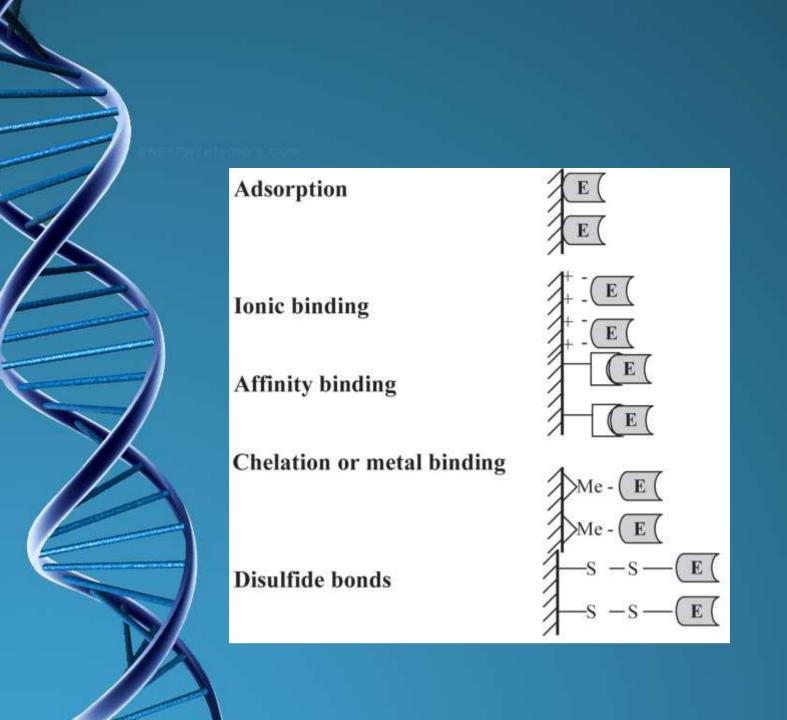
- Methods of Irreversible Enzyme
 Immobilization
 - The concept of irreversible immobilization means that once the biocatalyst is
- attached to the support it cannot be detached without destroying either the biological
- common procedures of irreversible
- enzyme immobilization are covalent coupling, entrapment or
- micro-encapsulation, and cross-linking

4.1. Formation of Covalent Bonds

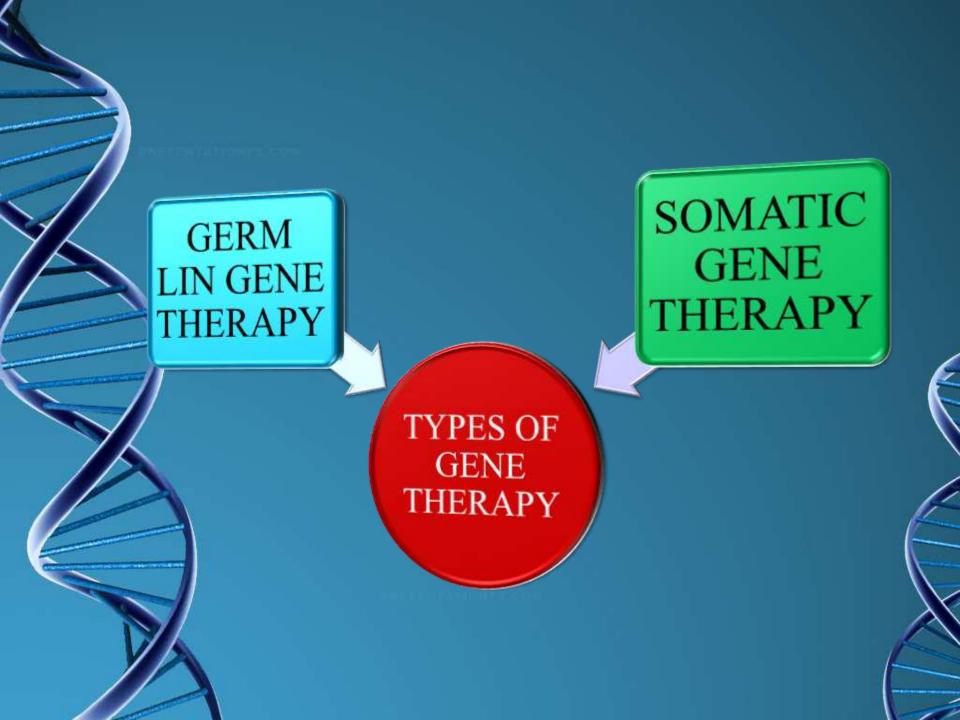
- Immobilization of proteins by methods based on the formation of covalent
- bonds are among the most widely used.

 An advantage of these methods is that,
- because of the stable nature of the bonds formed between enzyme and matrix, the
- enzyme is not released into the solution upon use. However, in order to achieve









Germ line gene therapy:

Therapeutic genes transferred into the **germ** cells.

- Eg. Genes introduced into eggs and sperms.

It is heritable and passed on to later generations.

For safety, ethical and technical reasons, it is not being attempted at present.

Somatic line gene therapy:

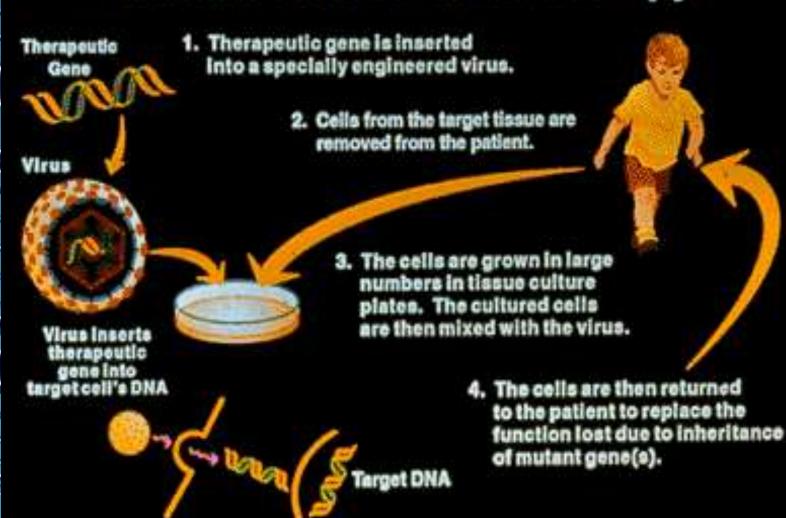
- Therapeutic genes transferred into the **somatic** cells.
- Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc.
- > Will not be inherited later generations.
- At present all researches directed to correct genetic defects in somatic cells.
- TYPES
 - Ex vivo
- −**In**/vivo

<u>Ex vivo gene transfer</u>:

Few defective cells removed from patient and grown in the laboratory.

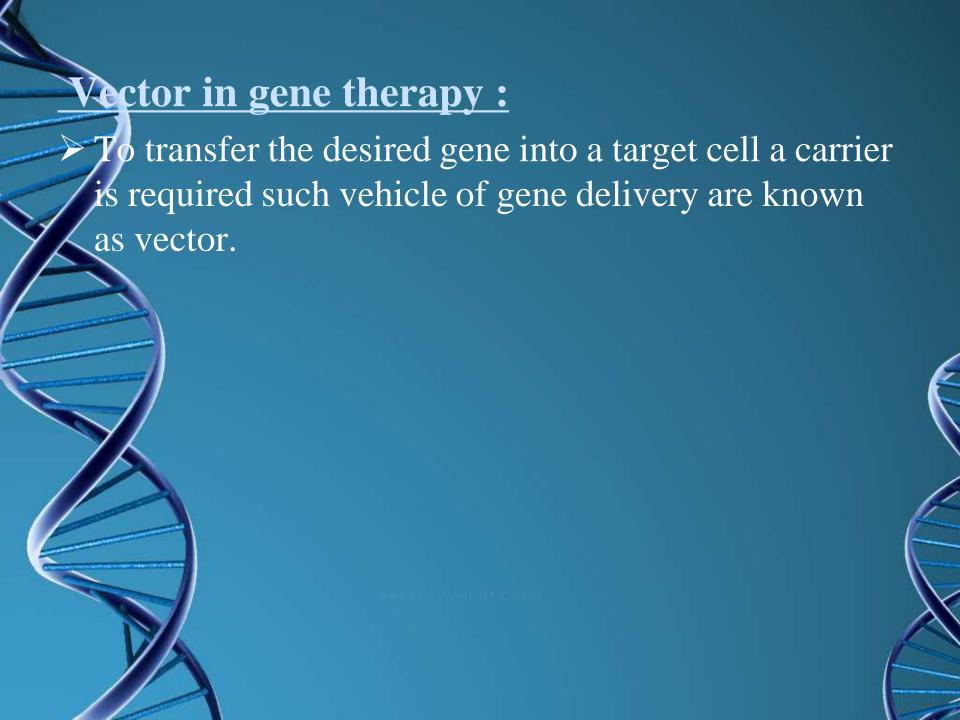
- The cells are exposed to the virus that is carrying the desired gene. The virus enters the cells and inserts the desired gene into the cell's DNA.
- The cells grow in the laboratory and then returned to the patient by injection into a vein

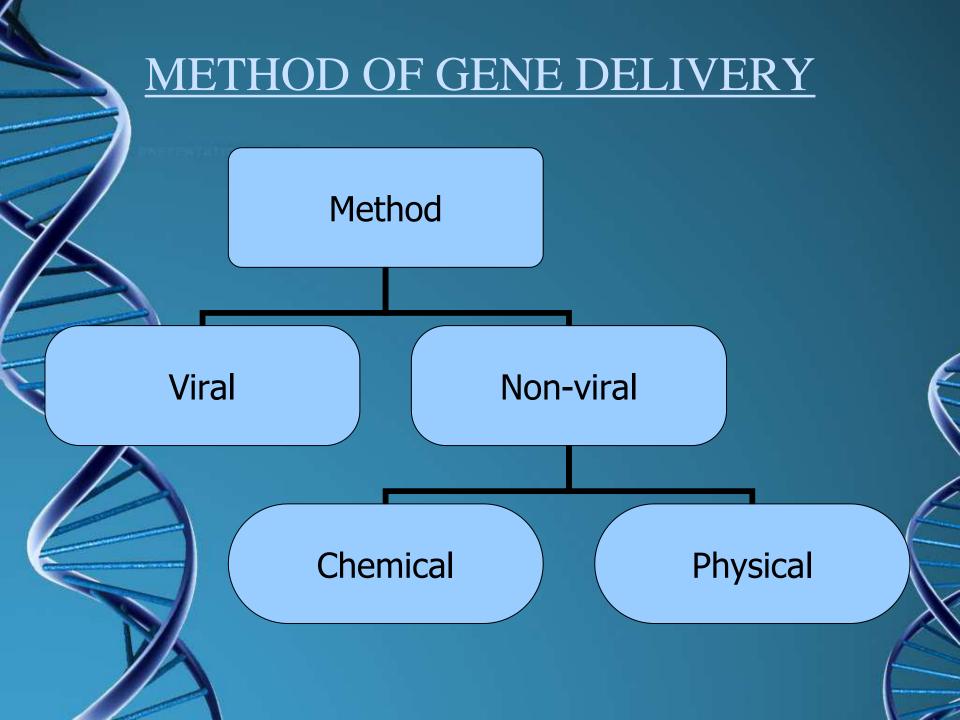
Human ex vivo Gene Therapy



<u>In vivo gene transfer :</u>

- Direct delivery of therapeutic gene into target cell into patient body.
- Carried by viral non viral vector system.
- It can be only possible option in patient where individual cell cannot be cultured in vitro on sufficient member.
- Ex: Brain cell.





Why viruses ???

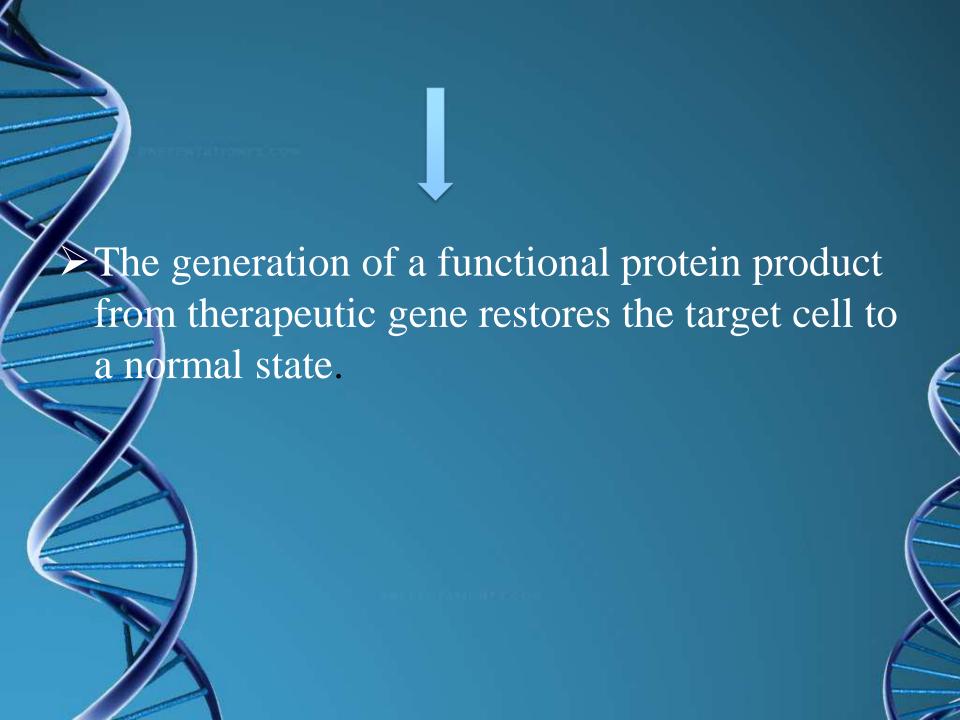
- High concentration of virus allowing many cells to be infected or transduced.
- Convenience and reproducibility of production.
- Ability to transduce dividing and non-dividing cells.
- Ability to integrate into a site-specific location in the
 - host chromosome, or to be successfully maintained as
 - stable episome.
- > Ability to target the desired type of cell.

Mechanism of viral gene delivery

Viruses genetically altered to carry normal human ONA.

Target cell such as the patient's liver or lung cells are infected with the viral vector.

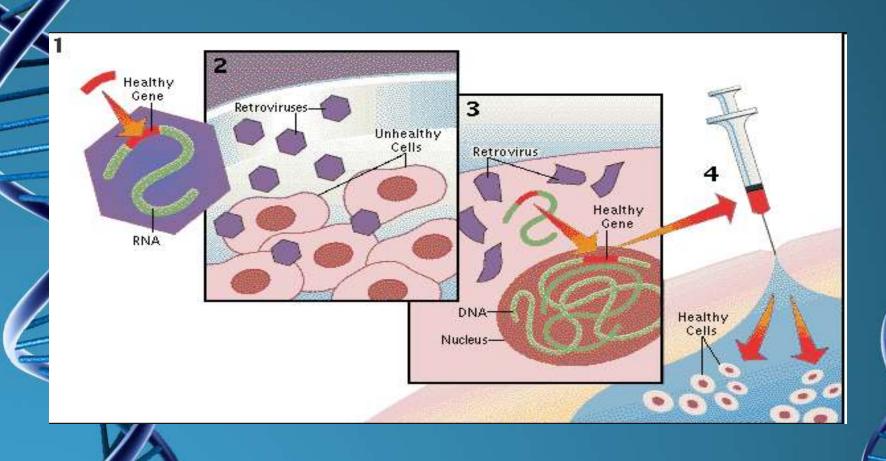
The viral vector then unload its genetic material containing the therapeutic human gene into the target



Retroviruses

- Genetic material in form of RNA, while of host, it is DNA
- RNA molecule must produce a DNA copy before it can be considered part of the genetic material of the host cell
- Equipped with enzyme reverse transcriptase.
- Host cell now contains a new gene, multiplies, restores function.

RETROVIRUSES CARRYING HEALTHY GENE ARE MIXED WITH UNHEALTHY CELLS TAKEN FROM A PATIENT. RETROVIRUSES INFECT UNHEALTHY CELLS WITH HEALTHY GENE, ADDING THE GENE TO PATIENT'S DNA. HEALTHY CELLS THEN INJECTED BACK TO THE PATIENT..



Disadvantages of Retroviruses

- Can only invade cells that divide often, therefore blood cells, skin cells and many other tissues can not be invaded by this vector.
- Do not insert their genetic material in any specific places, but in the middle of an important gene. The important gene could become defective, stop functioning and could do more harm than good...... (insertional mutagenesis)
 - e.g. leukemia in 10 patients
 - Immune rejection

Non viral methods of gene delivery

Direct introduction of therapeutic cDNA into target cells.

Advantage of low host immunogenicity.

Can be used only with certain tissues and requires large amount of DNA.

Physical methods

Injection of naked DNA

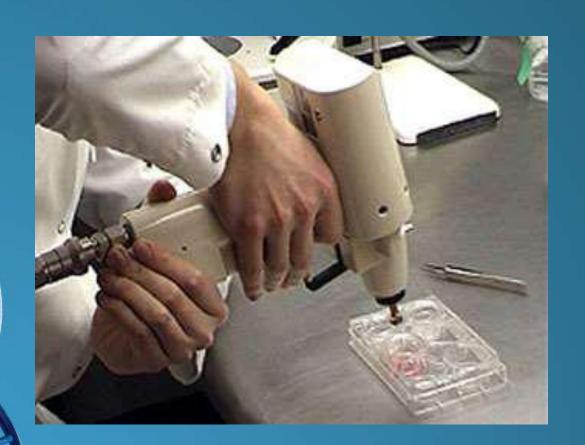
- IM injection of naked DNA plasmid.
- Low success rate.

Electroporation

- -Pulses of high voltage to carry DNA across membrane.
- Efficient and works across wide range of cell types

<u>Gene gun :</u>

- DNA coated with gold particles and loaded into device which generates force to achieve penetration of DNA into cells.

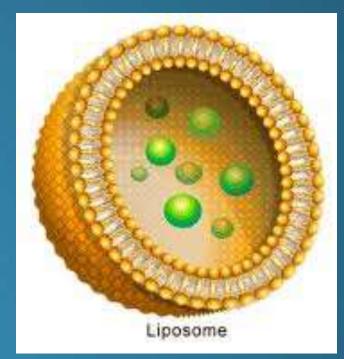


Chemical methods

Lipoplexes and polyplexes:

Cationic lipids due to their positive charge, used to condense negatively charged DNA so as to facilitate encapsulation of DNA into liposomes.

endocytosis of liposomes followed by lysis releases DNA into cytoplasm



Which diseases can be treated???

About 4000 diseases have been traced to gene disorders.

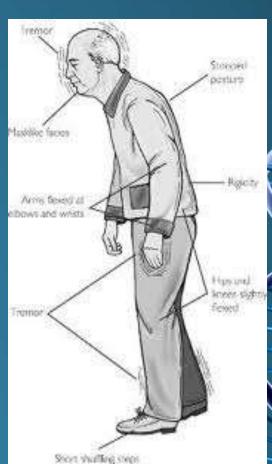
Current and possible candidates for gene therapy include cancer, AIDS, cystic fibrosis, Parkinson's, Alzheimer's disease, sickle cell disease, thalasemia etc.

Parkinson's disease

Loss of DA neurons in substantia nigra cells resulting into classical symptom.

In 2000, scientists have tried inserting genes controlling DA secretion into brain using liposomes coated in polymer.

Significant achievement because viral vectors are too big to cross BBB.



GENE THERAPY FOR CYSTIC FIBROSIS

- Fibrosis = Scarring, Cyst = Formation within the pancreas.
- Also known as MUCOVISCIDOSIS.
- Caused by Mutation in the gene of Protein named Cystic Fibrosis Transmemberane Regulator(CFTR).
- CFTR regulates the movement of-
 - Chloride
 - Sodium
 - across an epithelium

- Affects mainly Lungs and also Pancreas, Liver, Intestine.
- Main symptom--- Difficulty in breathing.
- CFTR was discovered more than 10 years ago and efforts are done to develop safe and effective vectors for introducing this gene into Respiratory Epithelium.
- After several Phase-1 clinical trial Results that mostly Adenovirus Vectors are used to transduce either nasal or pulmonary epithelium.
 - Disadvantage-
 - -Inefficient amount of transduced cells are transduce.

- Fransient nature of gene expression last for few days.
- Later AAV vectors for delivering CFTR are now in clinical trials.
- Preclinical studies demonstrated.
- Long term transgene expression.
- Long term safety and efficacy study of Adeno associated viral vector are under evaluation.
- Liposomal vectors are also under evaluation in Cystic Fibrosis.
- Liposomes may be mildly effective, but their activity does not last.

ADENOSINE DEAMINASE DEFICIENCY

- First genetic disorder to be clinically treated with gene therapy.
- Absence of Adenosine Deaminase in children leads to an accumulation of Deoxyadenosine triphosphate (toxic to Lymphocytes).
 - Patient with ADA develops recurrent life threatening infections due to cell-mediated and humeral immune responses.

- Standard therapy is bone marrow trans- plantation along with periodic infusions of PEG-coupled recombinant enzyme(PEG-ADA).
 - In the first clinical trial two patients were infused with periodic blood T lymphocytes that had been transduced with a retroviral vector containing the human ADA gene.
- One of these two patients had long term persistence of transduced T lymphocytes, while the other had a poor response.
- The responsive patient experienced amelioration of symptoms of the diseases and is living a normal life several years after treatment

