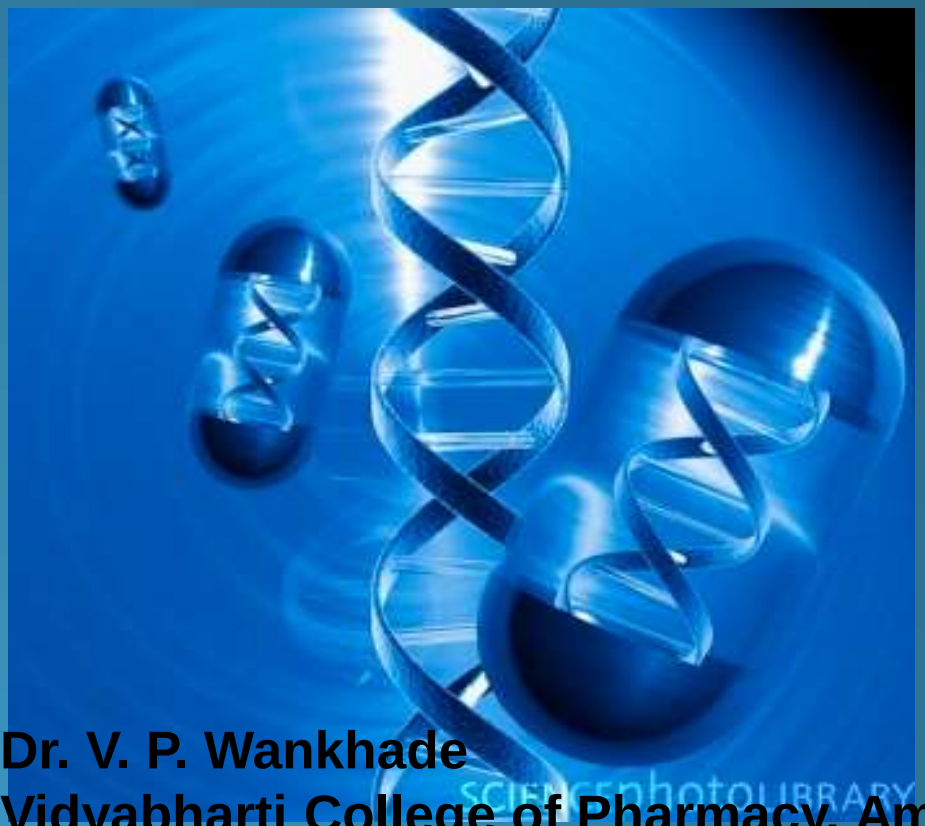
GENRAL INTRODUCTION AND POTENTIAL TARGET DISEASE FOR GENE THERAPY



Dr. V. P. Wankhade
Vidyabharti College of Pharmacy, Amravati

INTRODUCTION

- Def : Gene therapy is a treatment or cure for disorder caused by mutated gene.

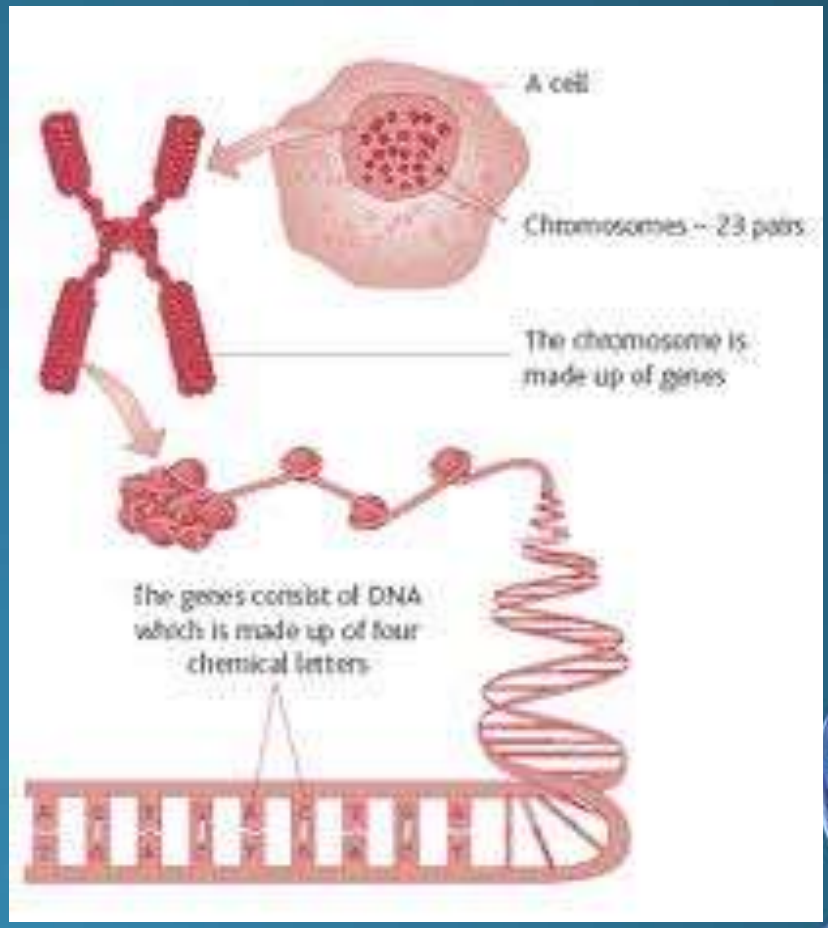
- It involve adding of a normally functioning copy of gene to enough affected cell to restore normal function.

- No two individual are same in this world, not even identical twins.

WHAT ARE GENE

- Biological unit of heredity.

- Determine obvious traits, such as hair and eye color, as well as more subtle characteristics such as the ability of the blood to carry oxygen.



- A gene is a part of DNA molecule, and humans have about 30,000 genes.

- Genes carry ‘instructions’ that allow the cells to produce specific proteins such as enzymes.

DNA → RNA → Proteins

- Only certain genes in a cell are active at any given moment. As cell mature, many genes become permanently inactive.

Mutation in genes

↓

Changes in codon sequence

↓

Altered transcription and translation

↓

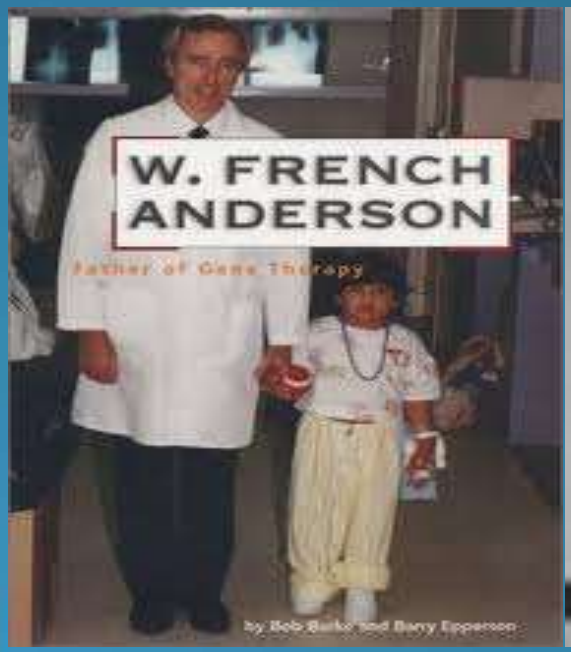
Defective protein synthesis

↓

Disease

- ✓ 4 approaches to correct defective gene :
- ➢ Normal gene may be inserted into nonspecific location within genome to replace defective one.
- ➢ Abnormal gene can be swapped for normal gene through homologous recombination.
- ➢ Abnormal gene could be repaired through reverse mutation.
- ➢ Regulation of particular gene could be altered.

FATHER OF GENE THERAPY



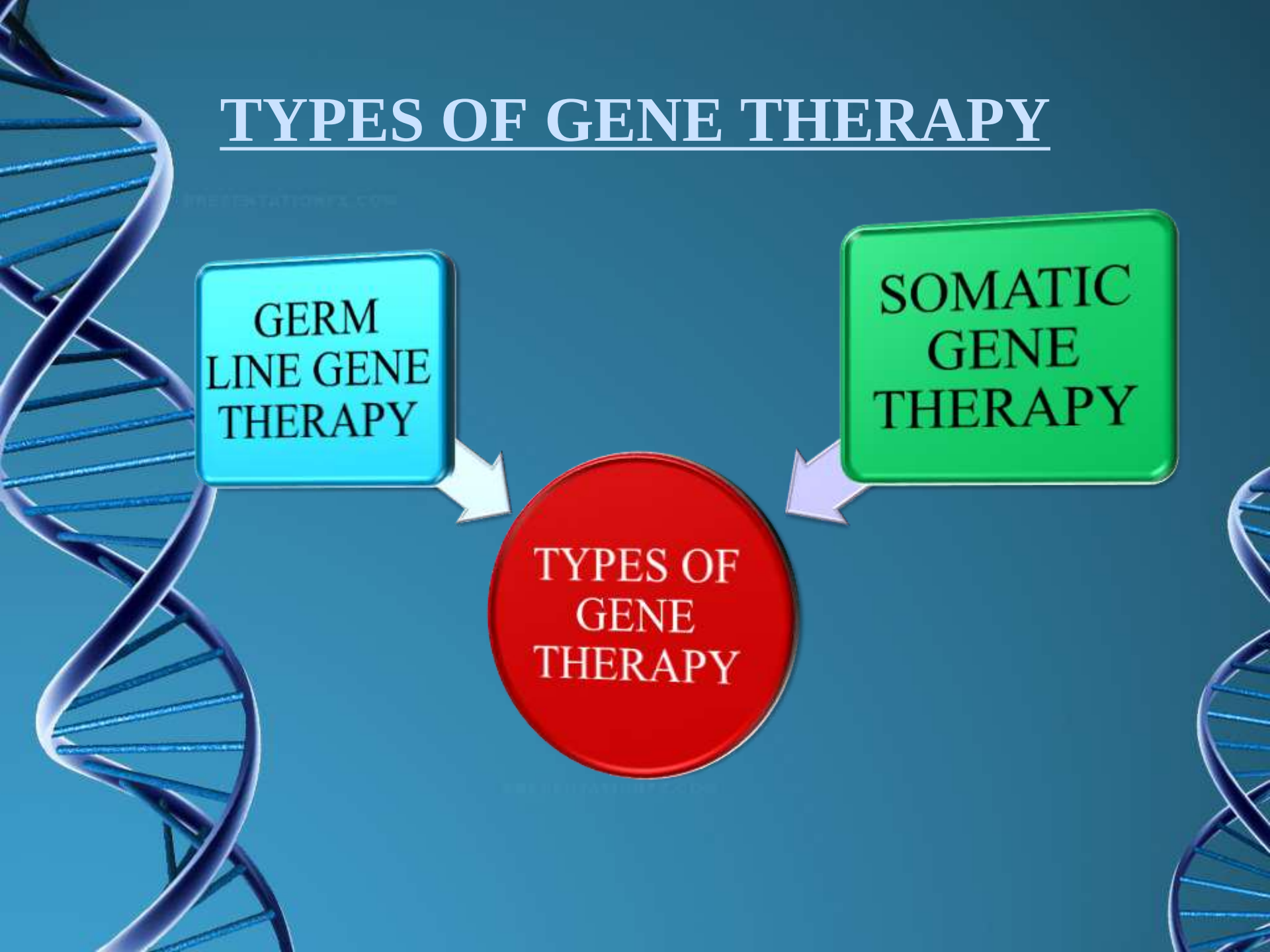
THE BEGINNING…!!!

The idea of GT was first Hypothesized by ANDERSON in Late Sixties.

- In the 1980s, Scientists began to look into gene therapy:
  - They would insert human genes into a bacteria cell.
  - Then the bacteria cell would transcribe and translate the information into a protein.
  - Then they would introduce the protein into human cells.

➢ - Ashanti DeSilva, 4 yrs, treated for SCID

TYPES OF GENE THERAPY



- **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.

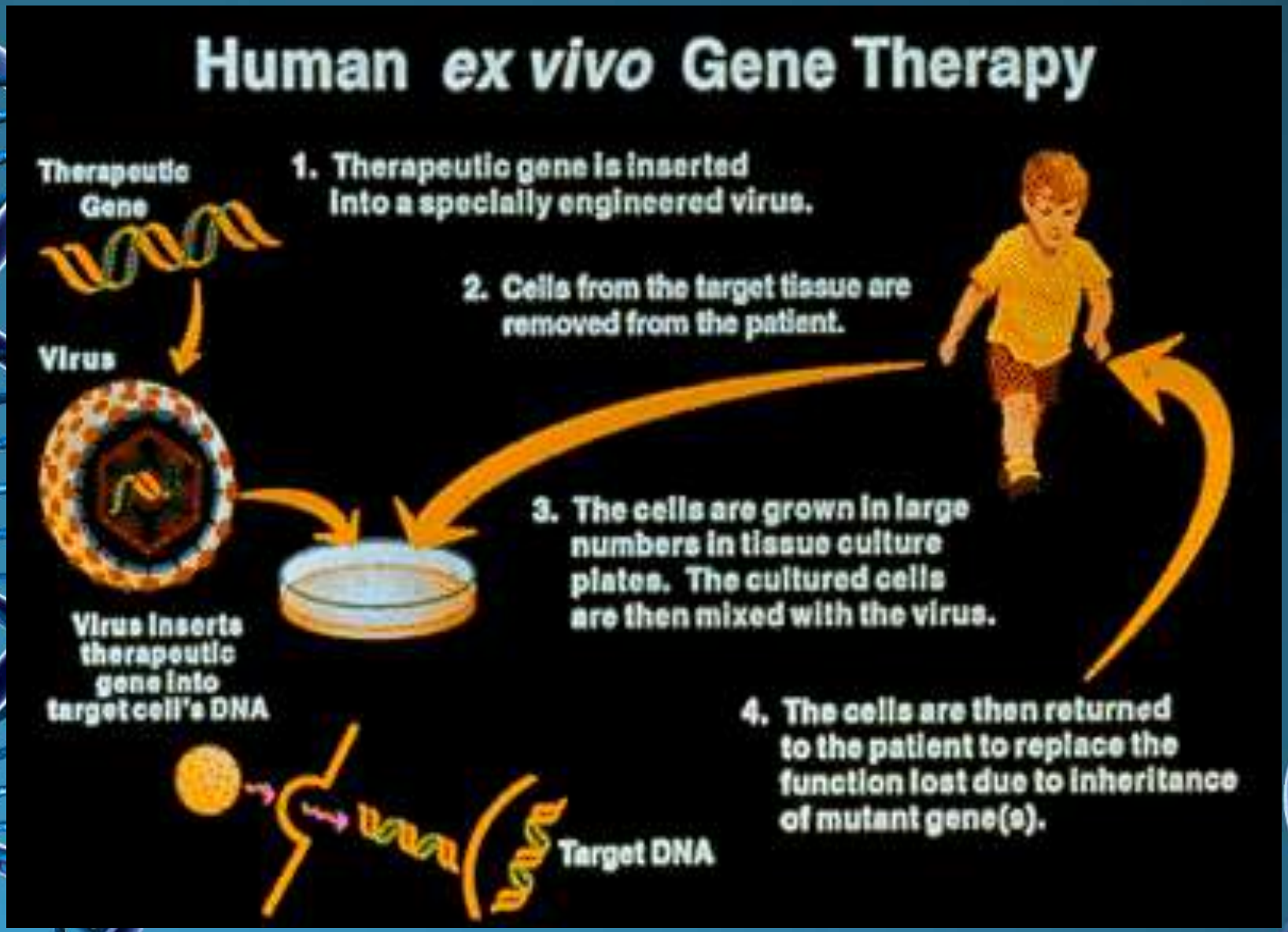
- **Somatic line gene therapy :**
  - Therapeutic genes transferred into the **somatic cells**.
  - Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc.
  - **Will not be inherited** later generations.
  - At present all researches directed to correct genetic defects in somatic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

- **Ex vivo gene transfer :**

➢ 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
Therapeutic Gene
1. Therapeutic gene is inserted into a specially engineered virus.
2. Cells from the target tissue are removed from the patient.
Virus
3. The cells are grown in large numbers in tissue culture plates. The cultured cells are then mixed with the virus.
Virus inserts therapeutic gene into target cell's DNA
4. The cells are then returned to the patient to replace the function lost due to inheritance of mutant gene(s).
Target DNA

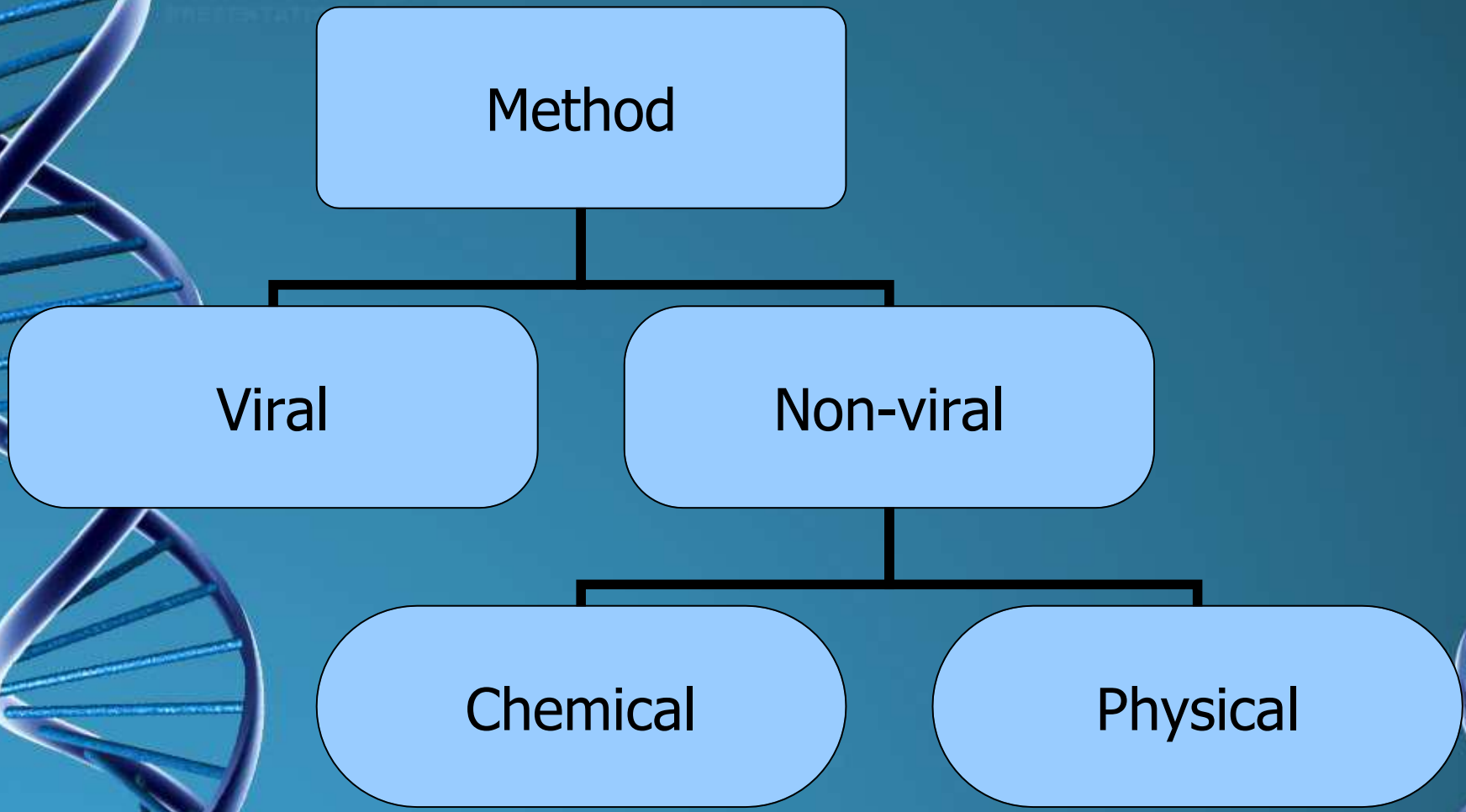
In vivo gene transfer :

- 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.

Vector in gene therapy :

- To transfer the desired gene into a target cell a carrier is required such vehicle of gene delivery are known as vector.

METHOD OF GENE DELIVERY



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 DNA.

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

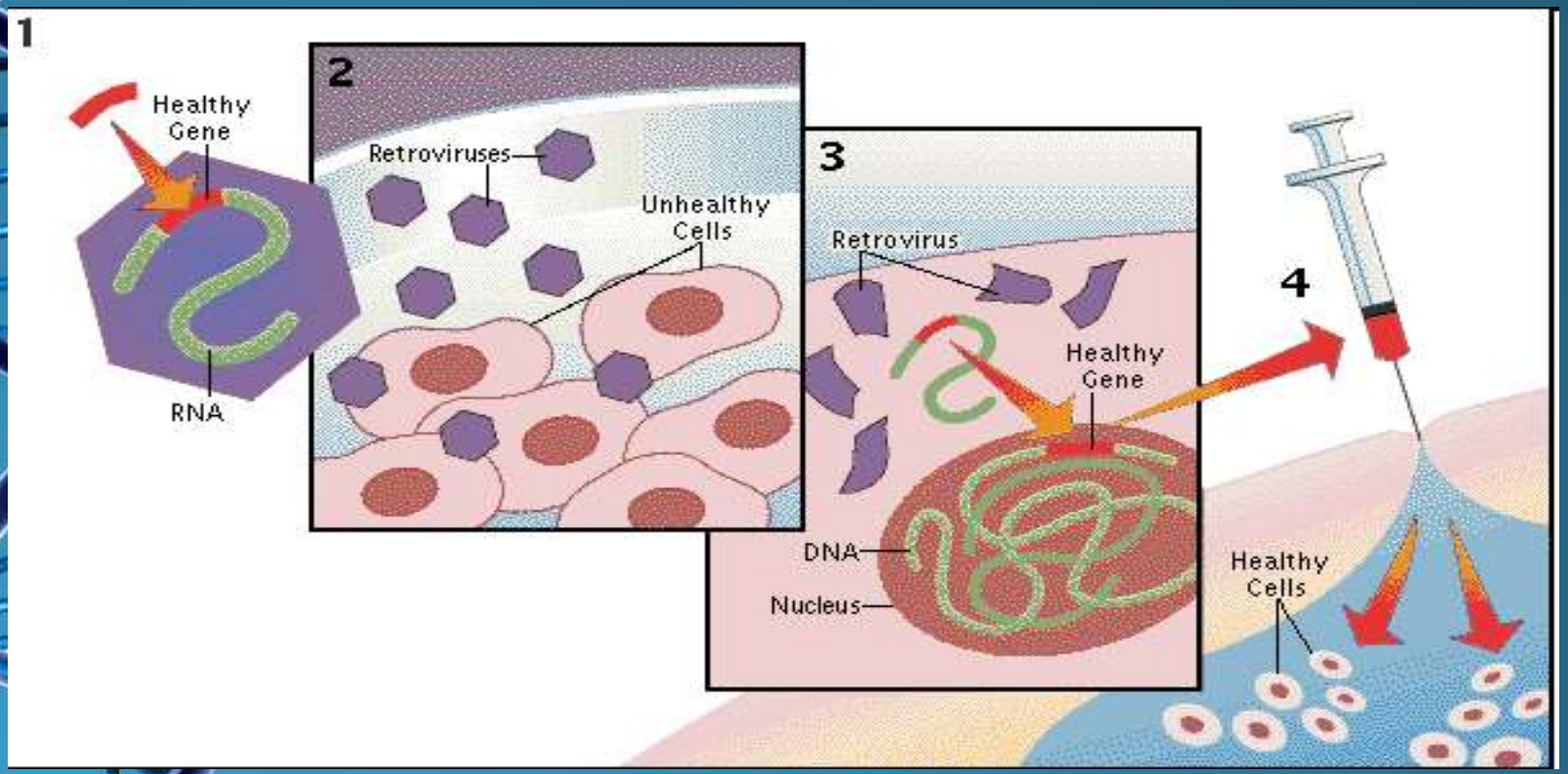
- The viral vector then unload its genetic material containing the therapeutic human gene into the target cell.

- The generation of a functional protein product from therapeutic gene restores the target cell to a normal state.

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

Gene gun :

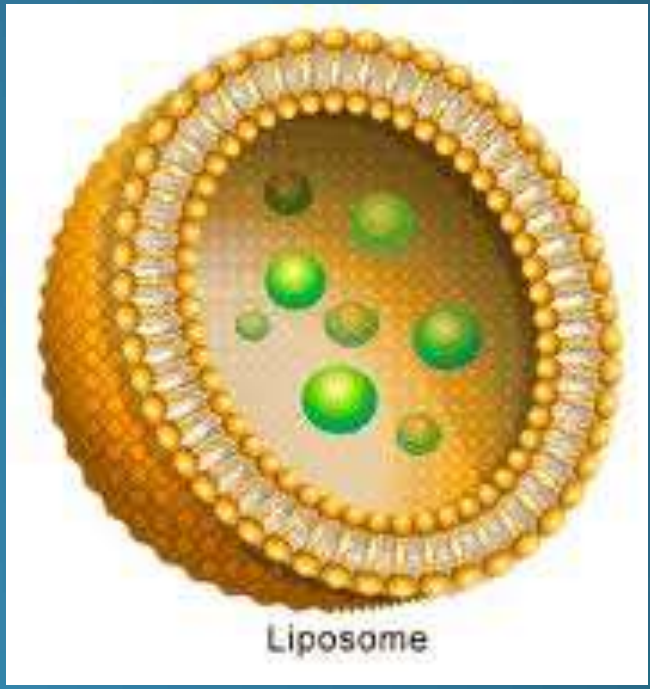
- DNA coated with gold particles and loaded into device which generates force to achieve penetration of DNA into cells.

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



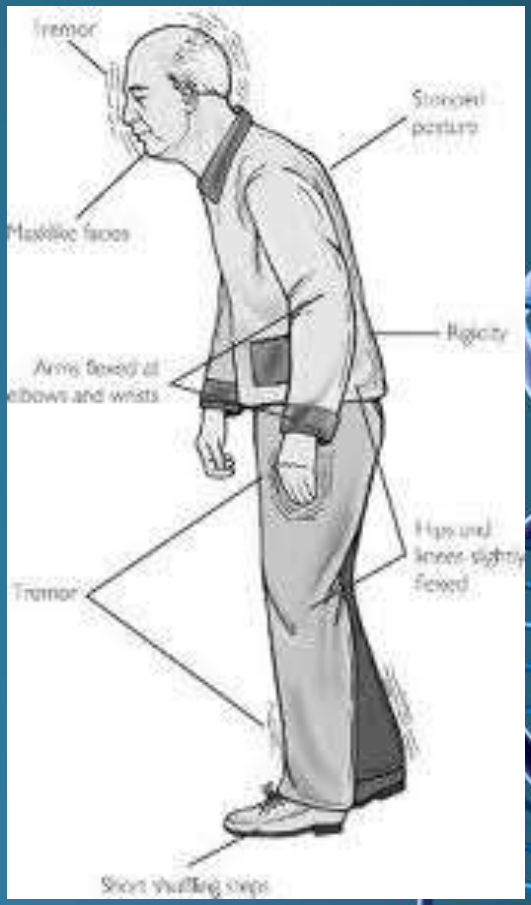
Which diseases can be treated ???

- 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

- CF→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.

- Transient 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


THANK YOU….!