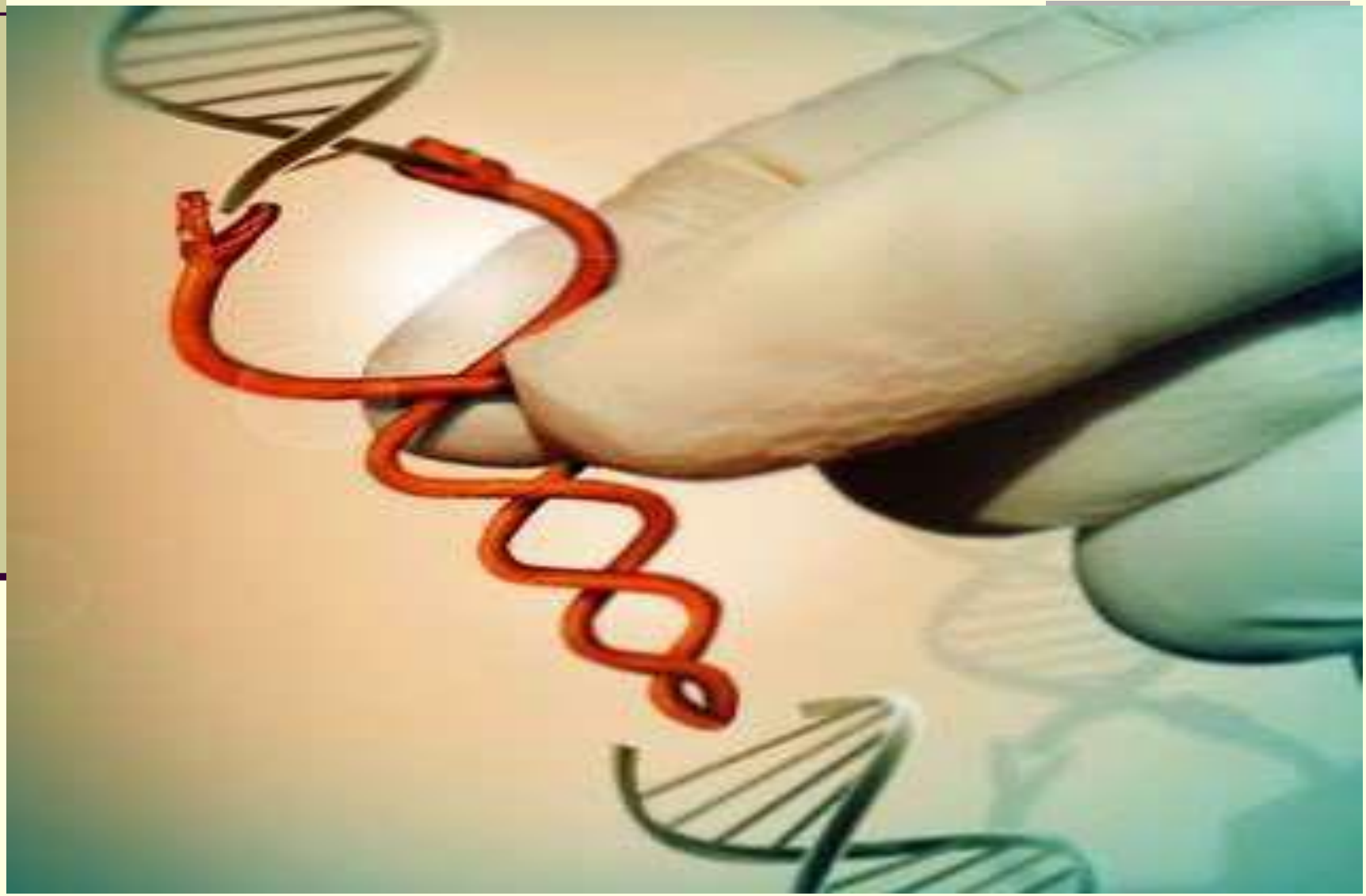
Lec.8 Gene Therapy



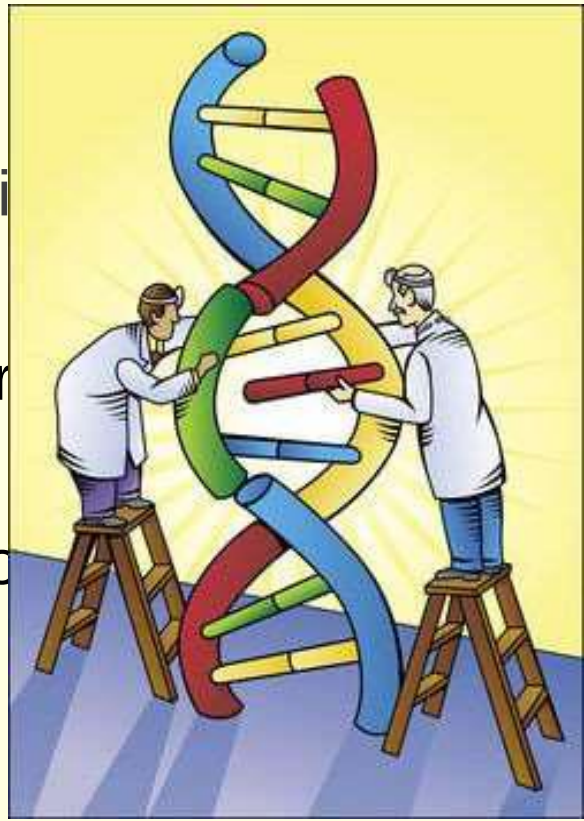
Contents

What is Gene Therapy

- Gene therapy is **the introduction of genes into cells** to prevent or cure a wide range of diseases.
- It is a technique for correcting defective genes responsible for disease development.

There are four methods:

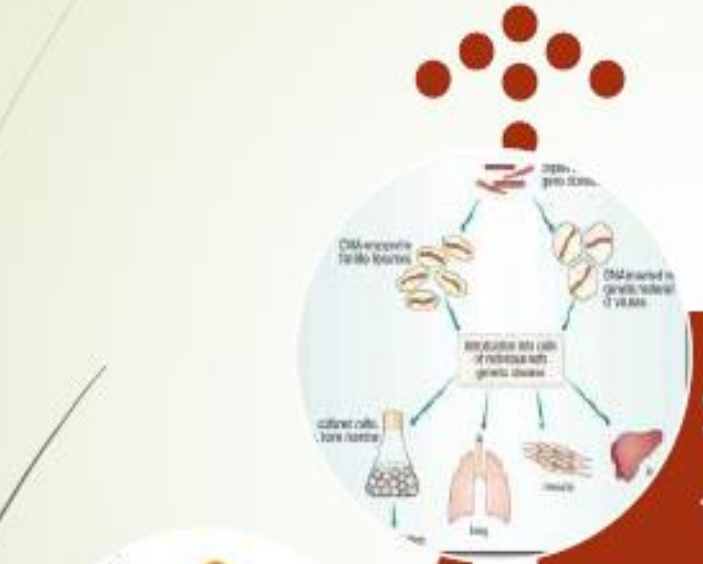
- **Replacing** a mutated gene that causes di
  with a healthy copy of the gene.
- **Inactivating**, A normal gene inserted to r
  non-functional gene.
- **Introducing a new gene** into the body to
  fight a disease.



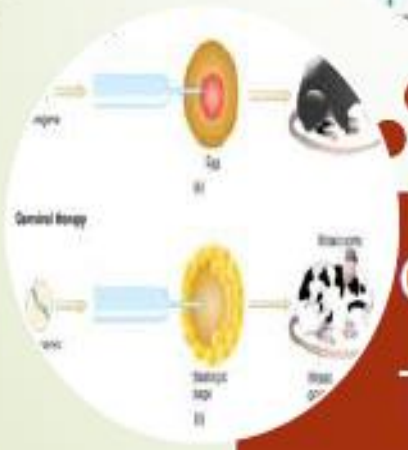
The First Case

- 1990, The first approved gene therapy experiment occurred on September 14, 1990 in US, when **Ashanti DeSilva /** Four-year-old was treated for **ADA-SCID**,(a severe immune system deficiency).
- Doctor William French Anderson removed her white blood cells, inserted the missing gene into the WBC, and then put them back into her blood stream. This supported her immune system.

Types of Gene therapy



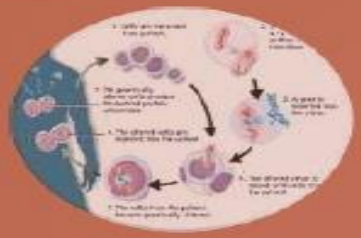
SOMATIC GENE THERAPY



GERM LINE GENE THERAPY

SOMATIC CELL GENE THERAPY	GERM LINE GENE THERAPY
▪ Therapeutic genes transferred into the **somatic cells.**	▪ Therapeutic genes transferred into the **germ cells.**
▪ Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc.	▪ Eg. Genes introduced into eggs and sperms.
▪ **Will not be inherited** later generations.	▪ **It is heritable** and passed on to later generations.
▪ At present all researches directed to correct genetic defects in somatic cells.	▪ For safety, ethical and technical reasons, it is not being attempted at present.

Types of somatic cell gene therapy



Ex vivo

- cells are modified outside the body and then transplanted back in again
- called ex vivo because the cells are treated outside the

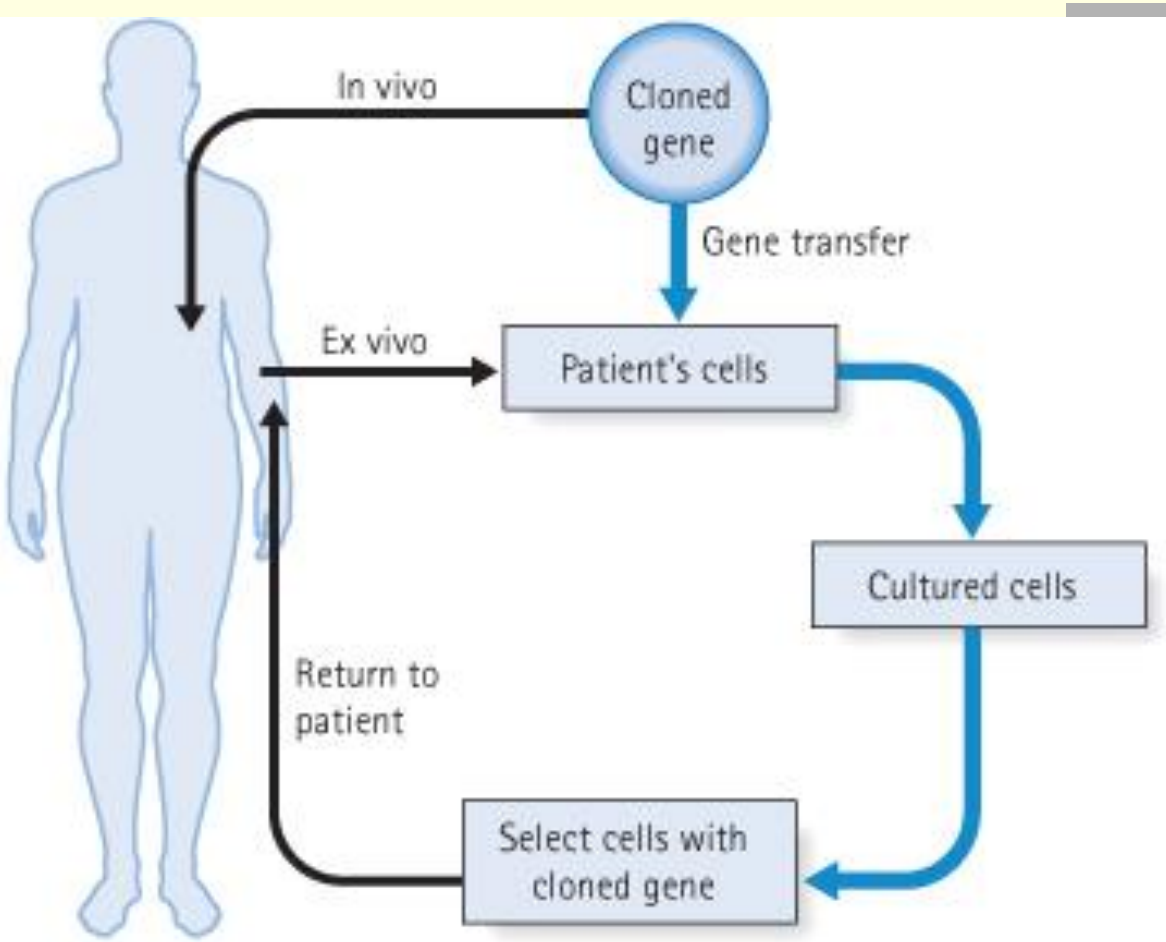
In vivo

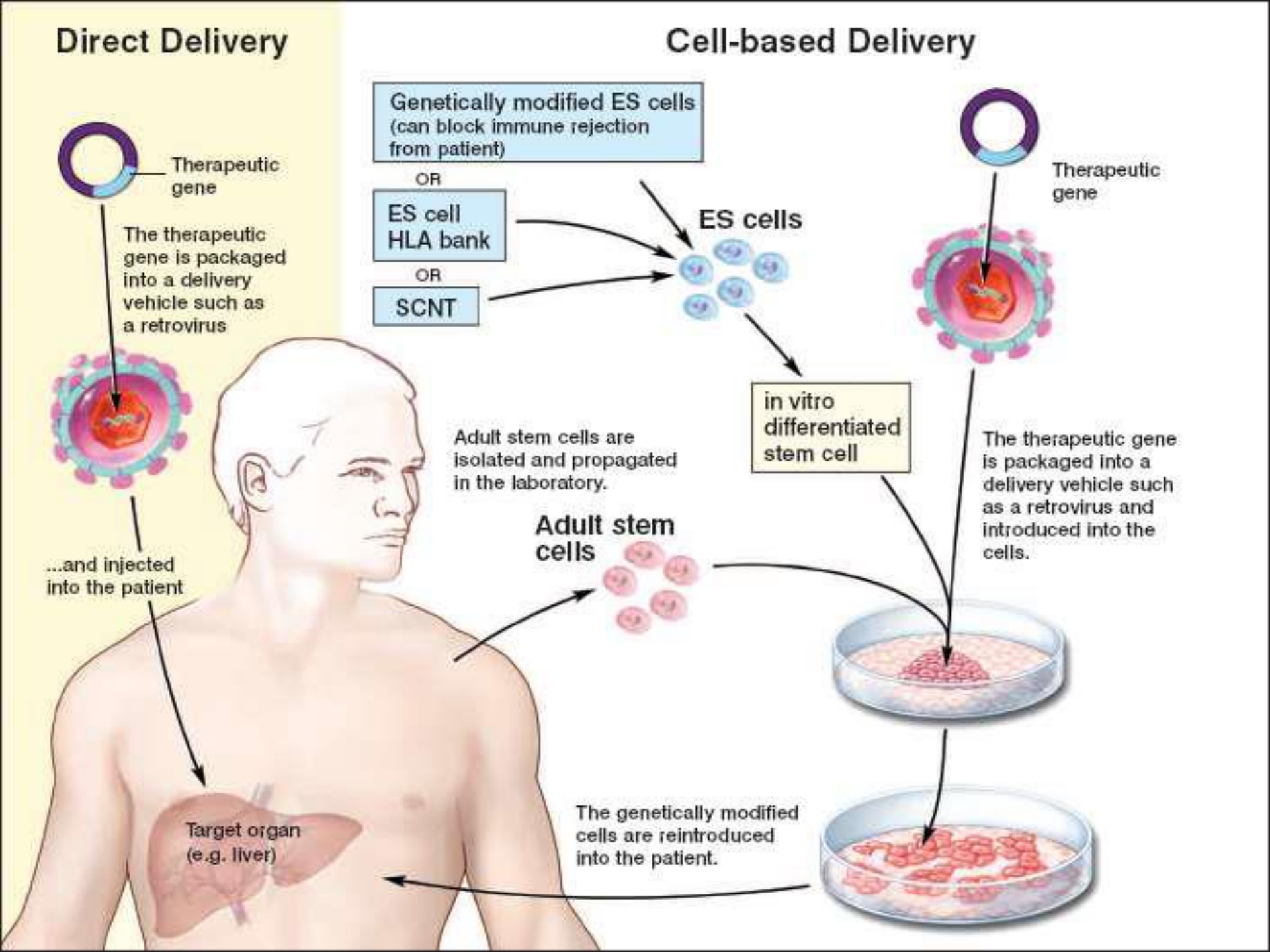
- genes are changed in cells when the cells are still in the body
- called in vivo because the gene is transferred to cells

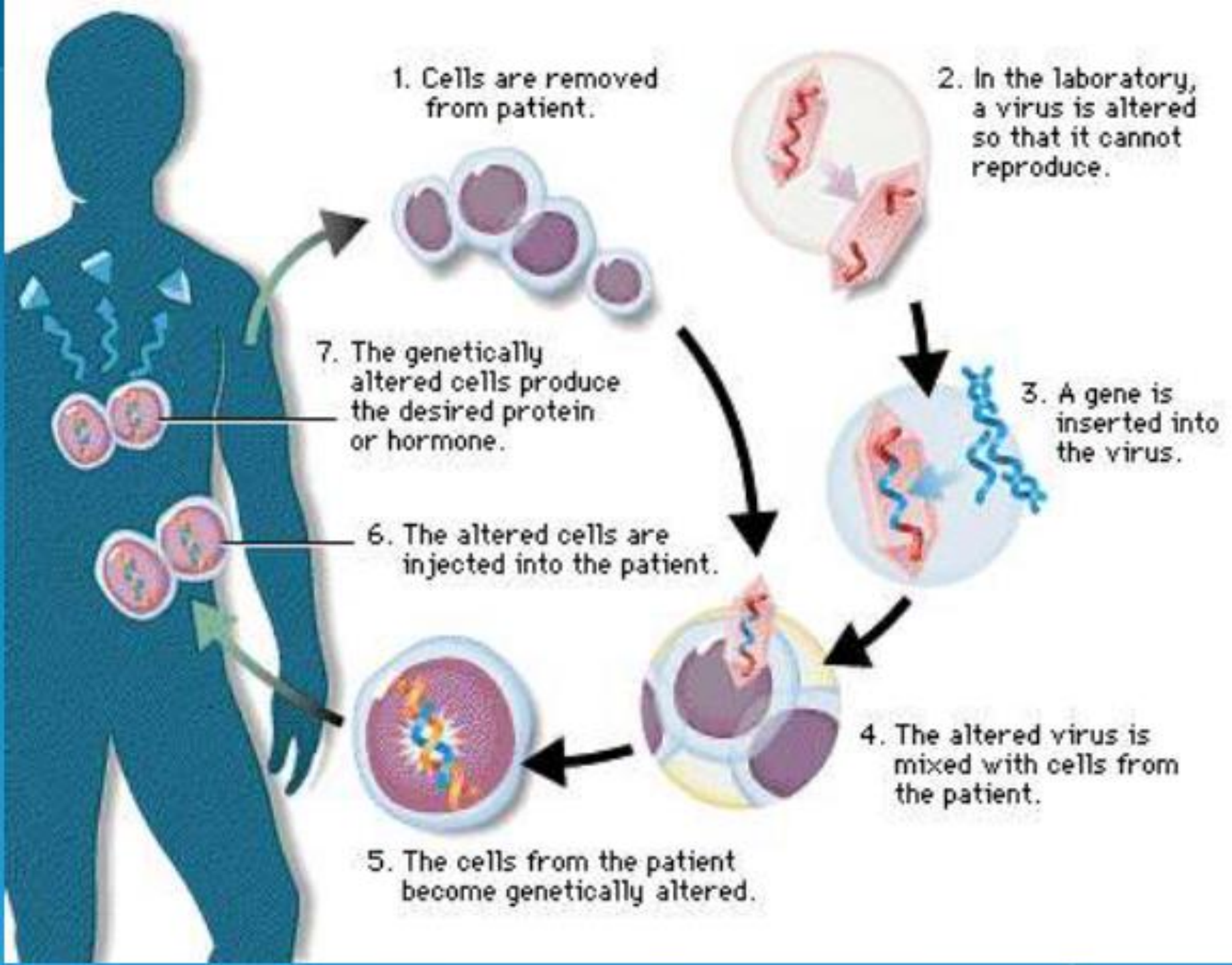
Ex vivo gene therapy:- transfer of genes to cultured cells and reinsertion.
Ex: SCID

In vivo gene therapy:- direct delivery of genes into the cells of a particular tissue in the body. Ex: cystic fibrosis
when ndividual cells cannot be cultured in sufficient numbers (e.g. brain cells).
Or when cultured cells cannot be reimplanted in patients effectively.

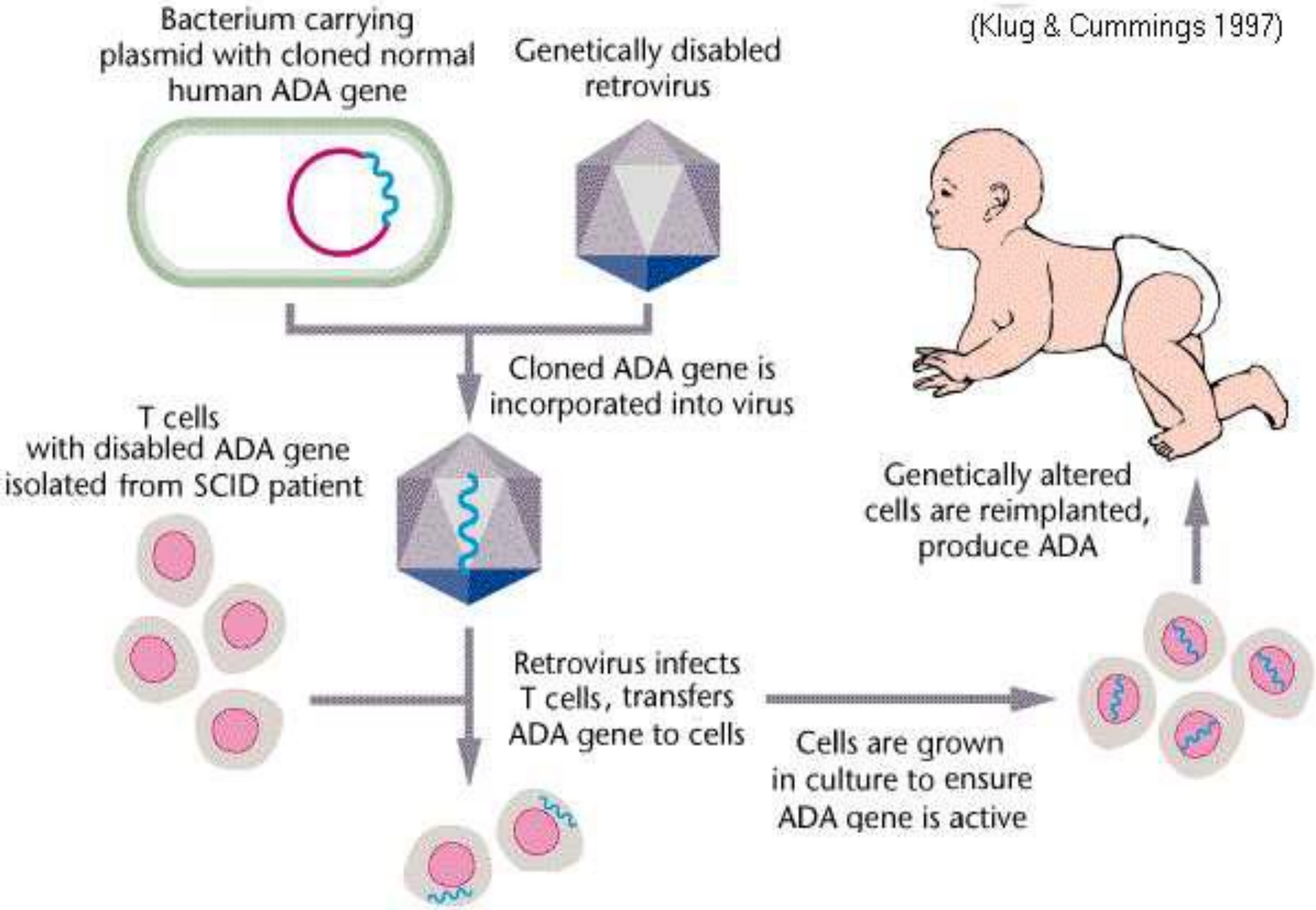
In vivo and ex vivo gene therapy



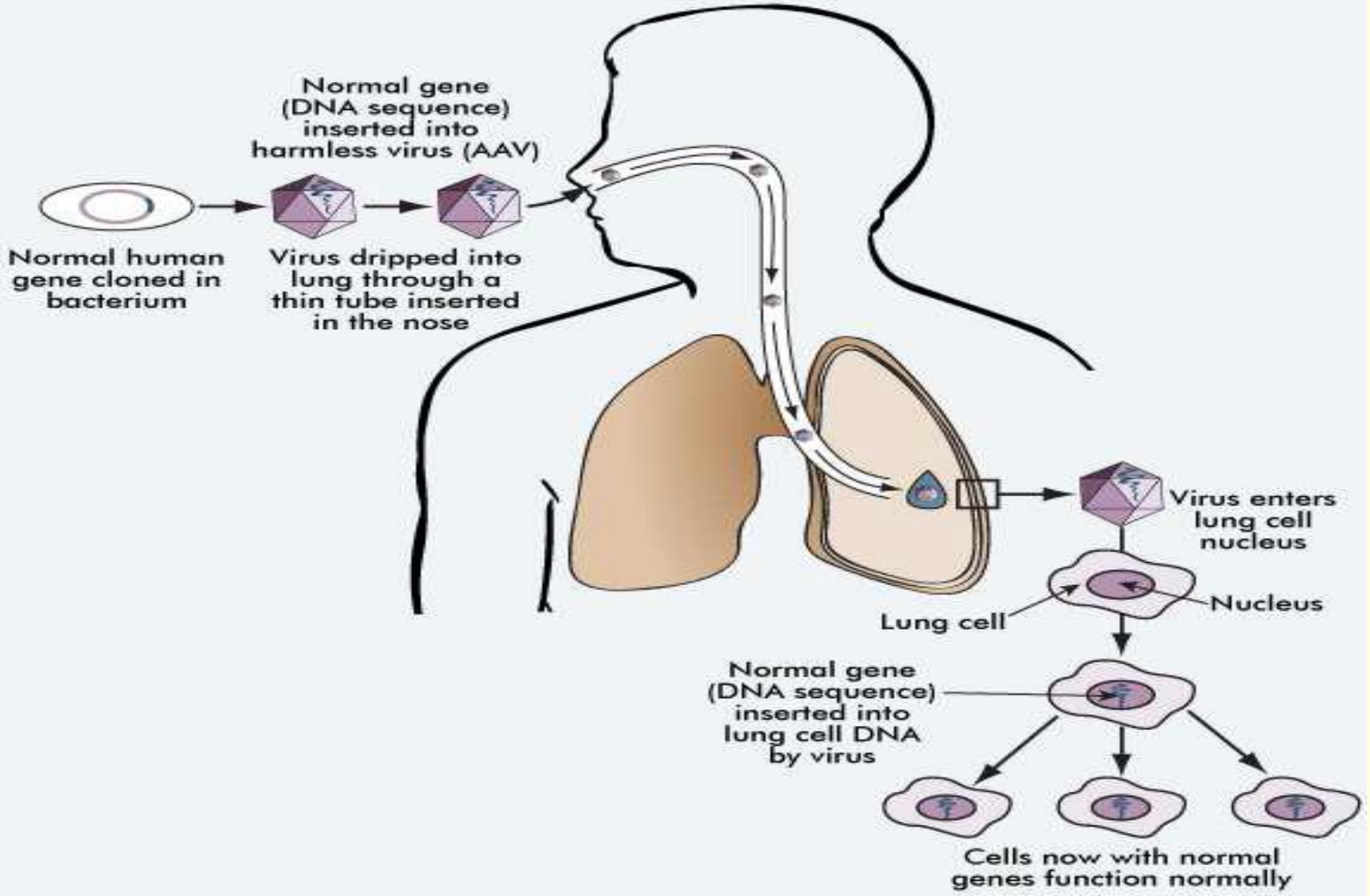

Direct Delivery
Cell-based Delivery
Genetically modified ES cells
(can block immune rejection
from patient)
OR
ES cell
HLA bank
OR
SCNT
ES cells
Therapeutic
gene
Therapeutic
gene
The therapeutic
gene is packaged
into a delivery
vehicle such as
a retrovirus
in vitro
differentiated
stem cell
Adult stem cells are
isolated and propagated
in the laboratory.
The therapeutic gene
is packaged into a
delivery vehicle such
as a retrovirus and
introduced into the
cells.
Adult stem
cells
...and injected
into the patient
Target organ
(e.g. liver)
The genetically modified
cells are reintroduced
into the patient.


1. Cells are removed from patient.
2. In the laboratory, a virus is altered so that it cannot reproduce.
3. A gene is inserted into the virus.
4. The altered virus is mixed with cells from the patient.
5. The cells from the patient become genetically altered.
6. The altered cells are injected into the patient.
7. The genetically altered cells produce the desired protein or hormone.

Ex vivo gene therapy for SICD

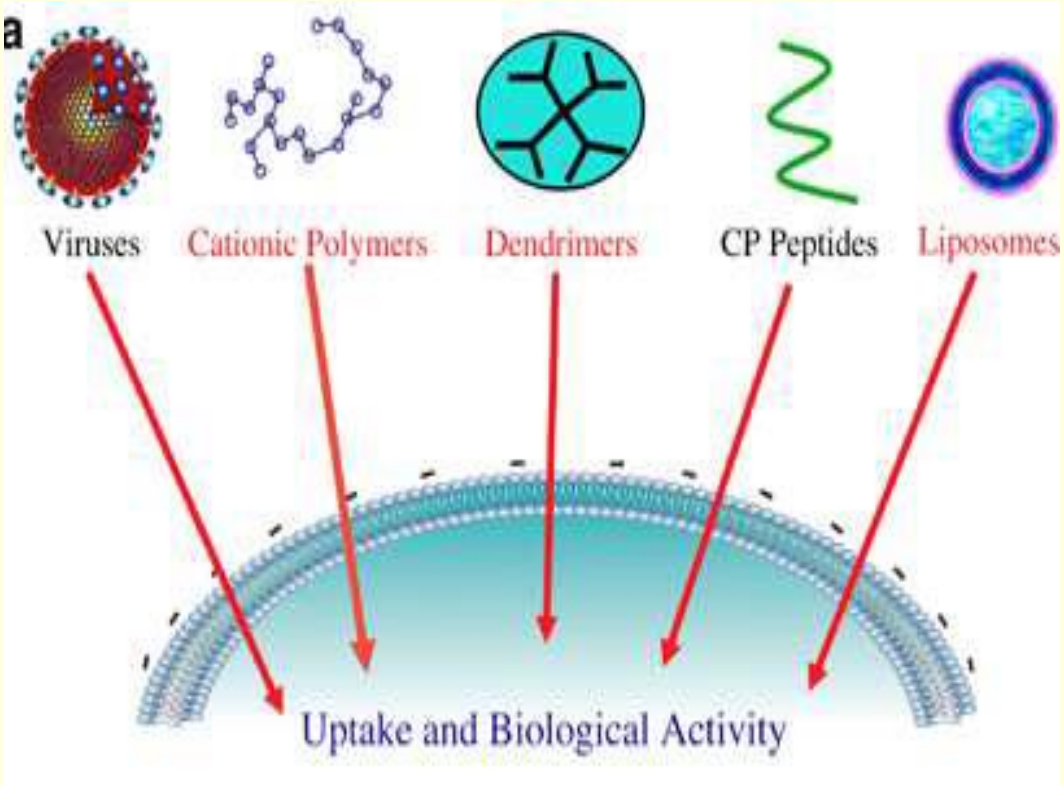


In vivo gene therapy for cystic fibrosis



Vector in gene therapy

- To transfer the desired gene into a target cell, a carrier is required. Such vehicles of gene delivery are known as **vectors**.
- 2 main classes
- **Viral** vectors
- **Non viral** vectors

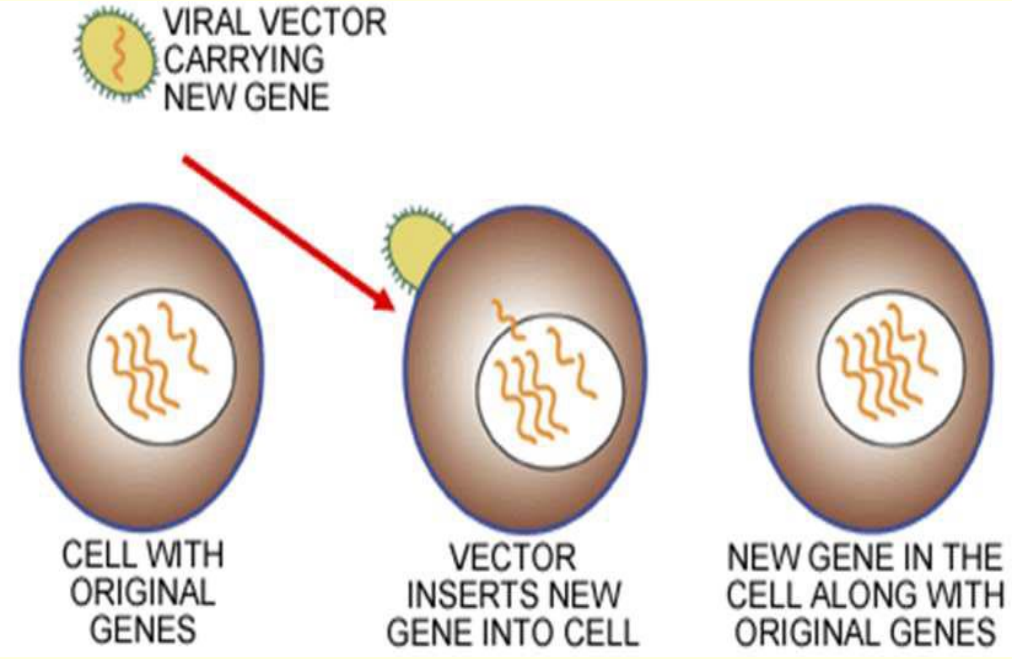


Viruses

- Viruses introduce their genetic material into the host cell as part of their replication cycle. Replicate by inserting their DNA into a host cell.
- remove the viral DNA and using the virus as a vehicle to deliver the therapeutic DNA.

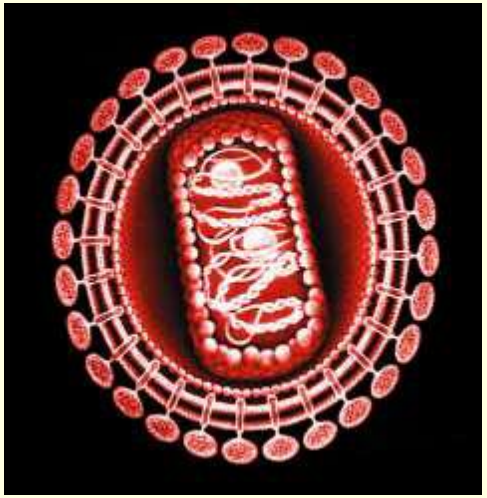
Types of viral vectors :

1-Retrovirus
2-Adenovirus
3-Adeno-Associated Virus
4-Herpes Simplex Virus



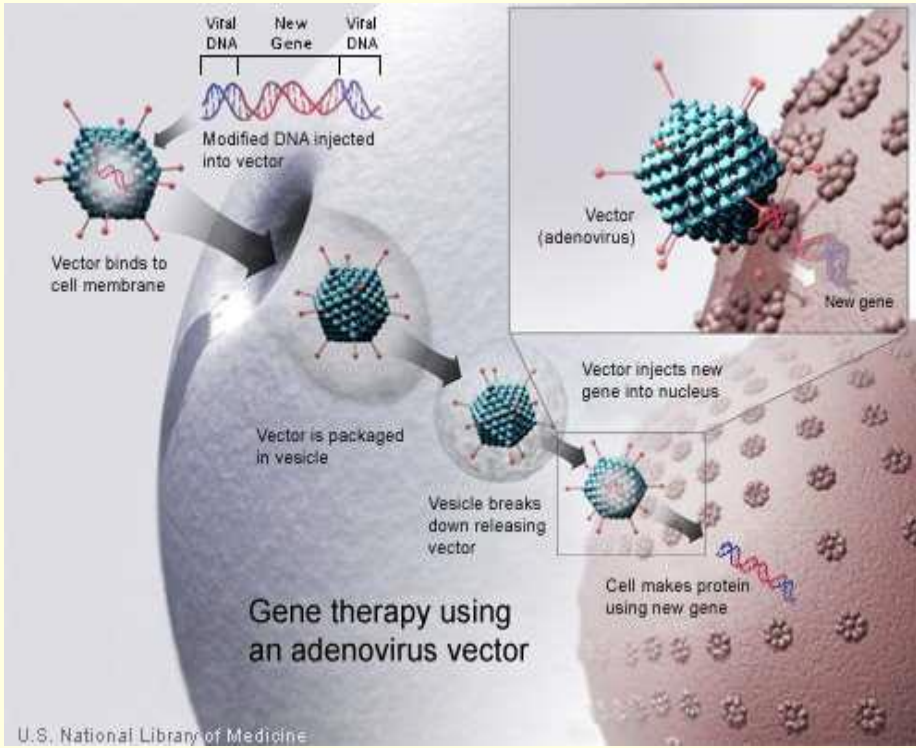
1-Retroviruses

- retroviruses have the ability to integrate into the host genome. integrals inserted the gene randomly because it has no specific site
- Can carry a DNA of size – less than 3.4kb
- Target cell – dividing
- May cause insertion mutagenesis



2- Adenoviruses

- Adenoviral DNA does not integrate into the genome and is not replicated during cell division.
- patients have already developed neutralizing antibodies which can inactivate the virus
- good vectors
- Target- non dividing cell.
- Eg. Common cold adenovirus.



3- Adeno-associated Viruses (AAV)

- It is a human virus that can integrate at a specific point into chromosome
- It is a single stranded, non pathogenic small DNA virus.
- AAV enters host cell, becomes double stranded and gets integrated into chromosome.
- AAV is not known to cause disease thus the virus causes a very mild immune response.
- Target- non dividing, dividing cells.
- Low information capacity
- hemophilia treatments, for example, a gene-carrying vector could be injected into a muscle, stimulating the muscle cells to produce Factor IX and thus prevent bleeding. patients have not needed Factor IX injections for more than a year.

4- Herpes Simplex Viruses

- The Herpes simplex virus is a human neurotropic virus Double stranded DNA viruses that natural infect a neurons
- . This is mostly examined for gene transfer in the nervous system.

Non-viral vectors

PURE DNA

- Direct introduction of pure DNA t into target tissue .
- DNA uptake by cells and expression rather low.
- Requires a lot of DNA have to be injected

Lipoplexes :

- Lipid DNA complexes; DNA surrounded by artificial lipid layer.
- Most of it gets degraded by lysosomes

DNA Molecule conjugates

- linking DNA to molecule that will bind to specific target cell receptors
- DNA is engulfed by cell membrane
- It avoids lysosomal breakdown of DNA

Human artificial chromosome

Trying to introduce a 47th chromosome

- Exist alongside the 46 others
- can carry a large DNA

METHODS OF GENE DELIVERY


Gene Gun

Physical methods

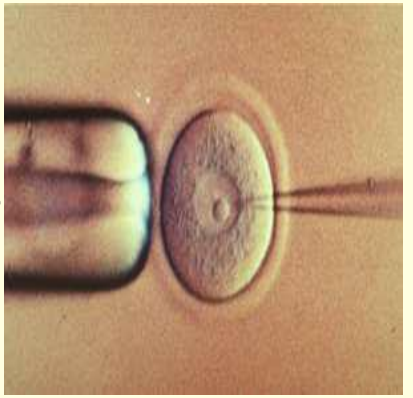
- Gene Gun
- Microinjection

using a glass micropipette to insert microscopic substances into a single living cell.

Chemical methods

- Using detergent mixtures
- Lipofection

used to inject genetic materials into a cell by liposomes Liposomes are artificial phospholipid vesicles used to deliver a variety of molecules including DNA into the cells.



Success case of gene therapy

- blindness of inherited condition
- Parkinson's
- 2006, X-linked chronic granulomatous disease
- 2007, Retinal disease
- 2010 beta-thalassemia
- 2011, HIV.
- 2016 , acute lymphobastic leukaemia (ALL)
- 2017, haemophilia A
- Research is still ongoing and the number of diseases that has
- been treated successfully by gene therapy increases.
- Cancer, immune disease, Diabetes Mellitus,etc.

Advantages

- remove and prevent hereditary diseases
- It is a possible cure for heart disease, AIDS and cancer.
- It gives someone born with a genetic disease a chance to life.
- It can be used to eradicate diseases from the future generations.

Disadvantages

- **Short Lived**
  - Hard to rapidly integrate therapeutic DNA into genome and rapidly dividing nature of cells prevent gene therapy from long time
  - Repeated treatment needed
    Genes “lost” when the cell goes through mitosis and Would have to e multiple rounds of therapy
- **Immune Response**
  - new things introduced leads to immune response
  - increased response when a repeat gene enters
- **Viral Vectors**
  - used as vectors for gene transfer may cause toxicity, immune responses, and inflammatory reactions in
- **Multigene Disorders**
  - Disorders caused by defects in multiple genes such as heart disease, blood pressure, Alzheimer's and diabetes cannot be treated effectively using gene therapy, you need to introduce more than one gene
- **May induce a tumor** if DNA integrated in the wrong place in the genome, for example in a tumor suppressor gene , it could induce a tumor