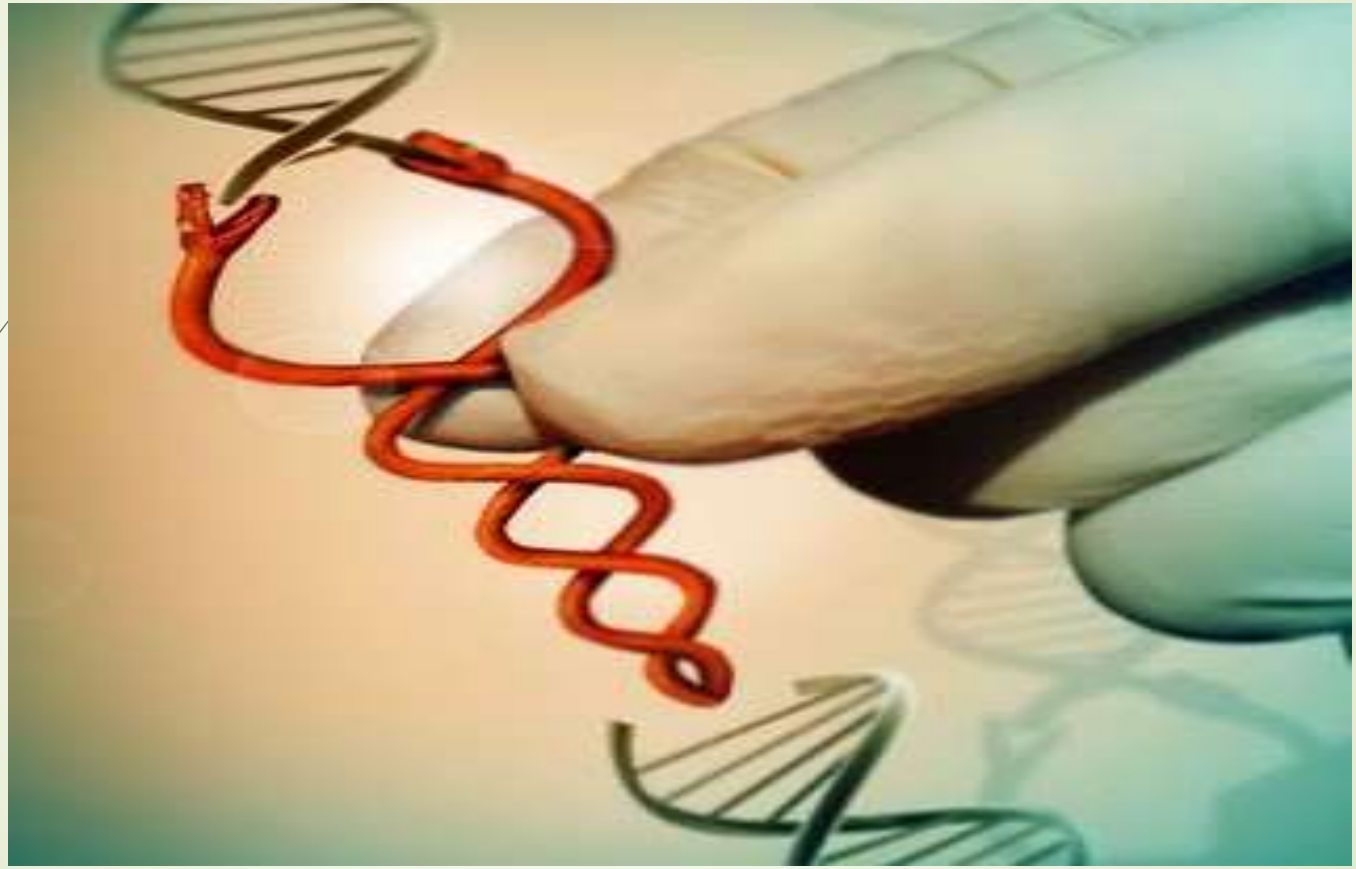
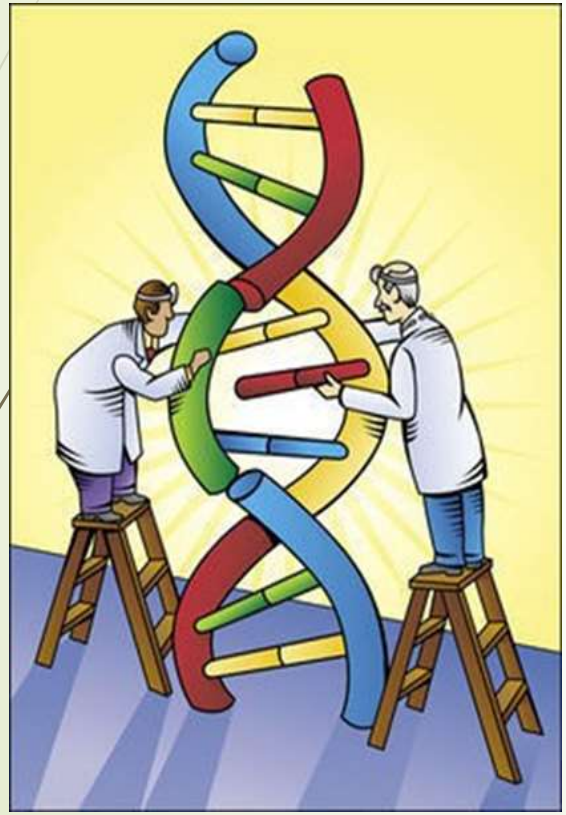
Gene Therapy



Contents

What is Gene Therapy?



- An approach of treating diseases by either modifying the expressions of an individual's genes or correction of abnormal genes.

Cont….

- This can be accomplished by:
- **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 help fight a disease.

History

- **1960's :** The concepts of Gene Therapy was introduced.
- **1972 :** Friedman and Roblin authored a paper in Science titled "Gene therapy for human genetic disease."
- **1984:** A retrovirus vector system was designed that could efficiently insert foreign genes into mammalian chromosomes.

- **1990**: The first approved gene therapy in the US took place on 14 September 1990, at the National Institutes of Health (NIH), under the direction of William French Anderson.
- Four-year-old Ashanti DeSilva received treatment for a genetic defect that left her with **ADA-SCID**, a severe immune system deficiency.

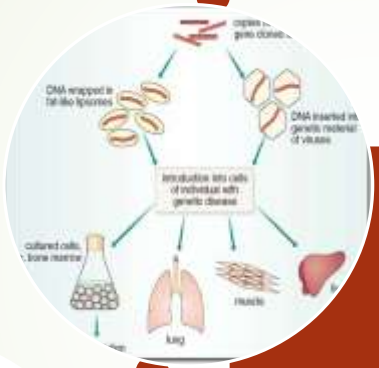
- **1992:** Doctor **Claudio Bordignon** performed the first procedure of gene therapy using hematopoietic stem cells as vectors to deliver genes intended to correct hereditary diseases.

- **1999:** Death of **Jesse Gelsinger** in a gene-therapy experiment

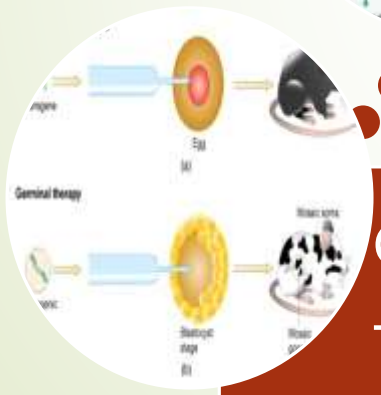
- **2003** : a research team inserted genes into the brain for the first time. They used liposomes which, unlike viral vectors, are small enough to cross the blood–brain barrier.
- **2006** : successful use of gene therapy to treat two adult patients for X-linked chronic granulomatous disease.
- **2007:** first gene therapy trial for inherited retinal disease

- **2010 :** an 18 year old male patient in France with beta-thalassemia major had been successfully treated.
- **2011:** Medical community accepted that it can cure HIV as in 2008, Gero Hutter has cured a man from HIV using gene therapy.
- **20011- 2015:** Research is still ongoing and the number of diseases that has been treated successfully by gene therapy increases.

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.
E.g.. Introduction of genes into bone marrow cells, blood cells, skin cells etc.	E.g.. 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

Cleaning Genes

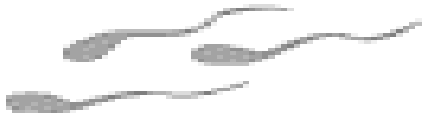
One way germline therapy may be done to ensure a man with a genetic disease doesn't pass it to his child:

1 Doctors remove the man's sperm-producing cells, which contain a defective gene.

2 A healthy gene is added to each cell to replace the defective ones.

3 The cells are put into mouse testes.

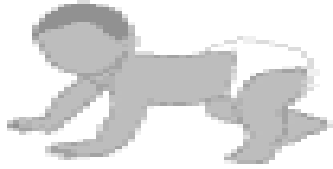
4 They mature inside the mouse and start producing healthy human sperm.



5 Those sperm, once tested, are used to fertilize a woman's eggs in a laboratory dish.

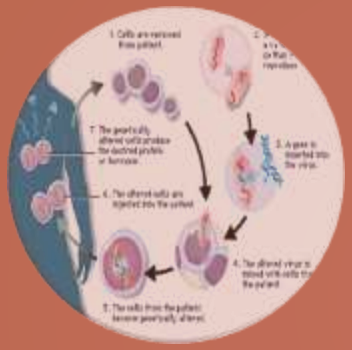
6 The resulting embryos are placed in a woman's womb.

7 She gives birth to a child whose genes are free from the father's disease.



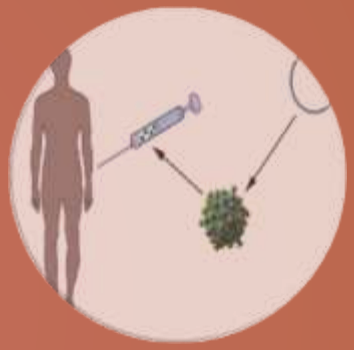
THE WASHINGTON POST

Types of somatic cell gene therapy



Ex vivo

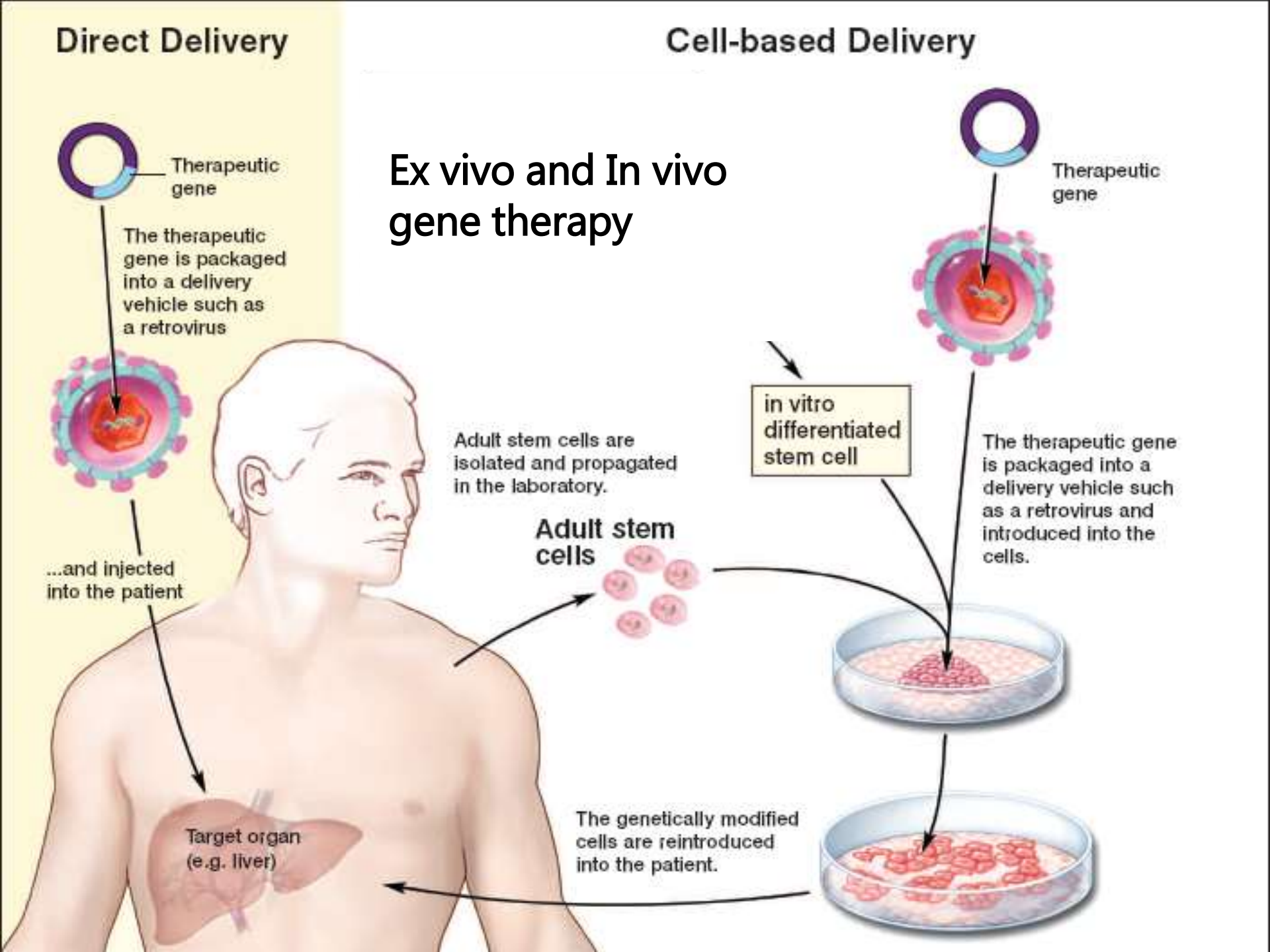
- cells are modified outside the body and then transplanted back in again
- called ex vivo because the cells are treated outside the



In vivo

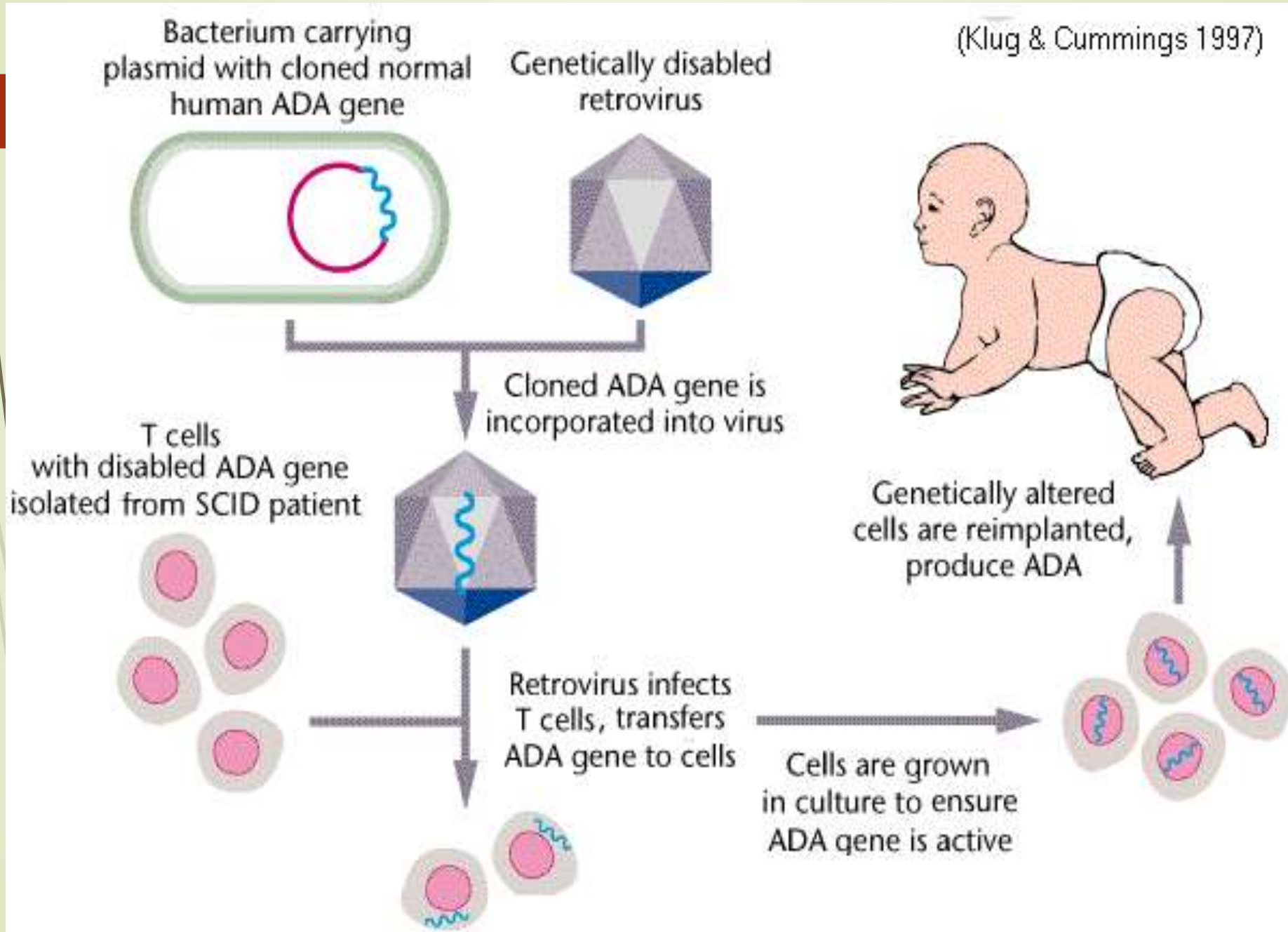
- genes are changed in cells when the cells are still in the body
- called in vivo because the gene is transferred to cells

Ex vivo and In vivo gene therapy



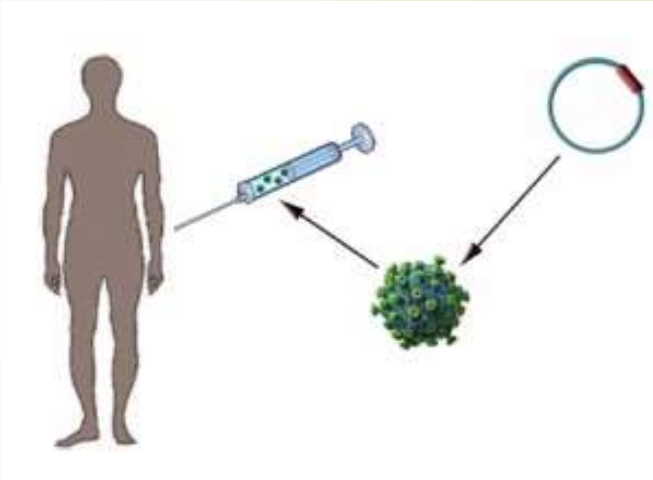
EXAMPLE OF EX VIVO GENE THERAPY

- 1st gene therapy – to correct deficiency of enzyme, **Adenosine deaminase (ADA).**
- Performed on a 4yr old girl Ashanthi DeSilva.
- Was suffering from **SCID- Severe Combined Immunodeficiency.**
- Caused due to defect in gene coding for ADA.
- Deoxyadenosine accumulate and destroys T lymphocytes.
- Disrupts immunity , suffer from infectious diseases and die at young age.


Bacterium carrying plasmid with cloned normal human ADA gene
Genetically disabled retrovirus
(Klug & Cummings 1997)
Cloned ADA gene is incorporated into virus
T cells with disabled ADA gene isolated from SCID patient
Genetically altered cells are reimplanted, produce ADA
Retrovirus infects T cells, transfers ADA gene to cells
Cells are grown in culture to ensure ADA gene is active

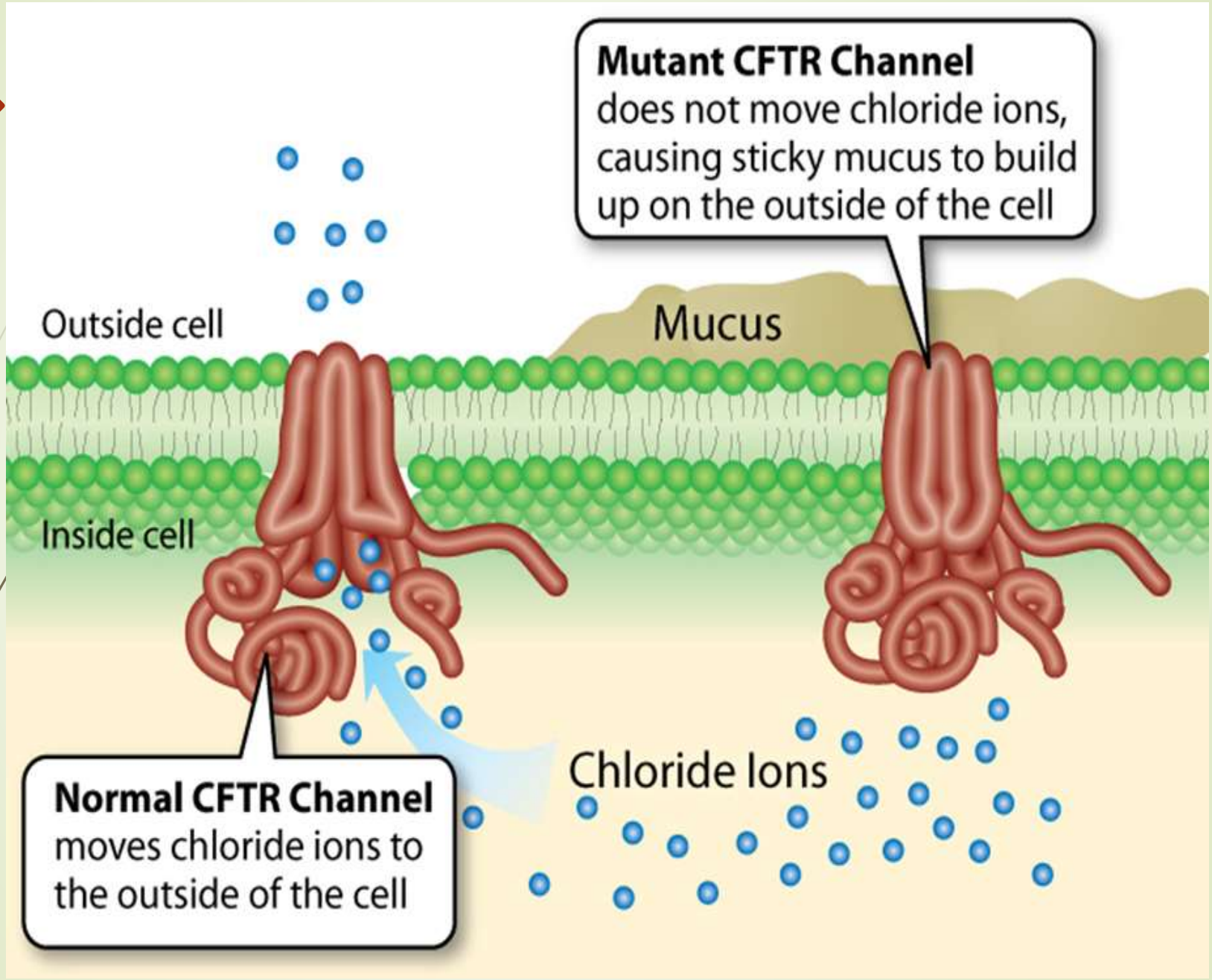
IN VIVO GENE THERAPY

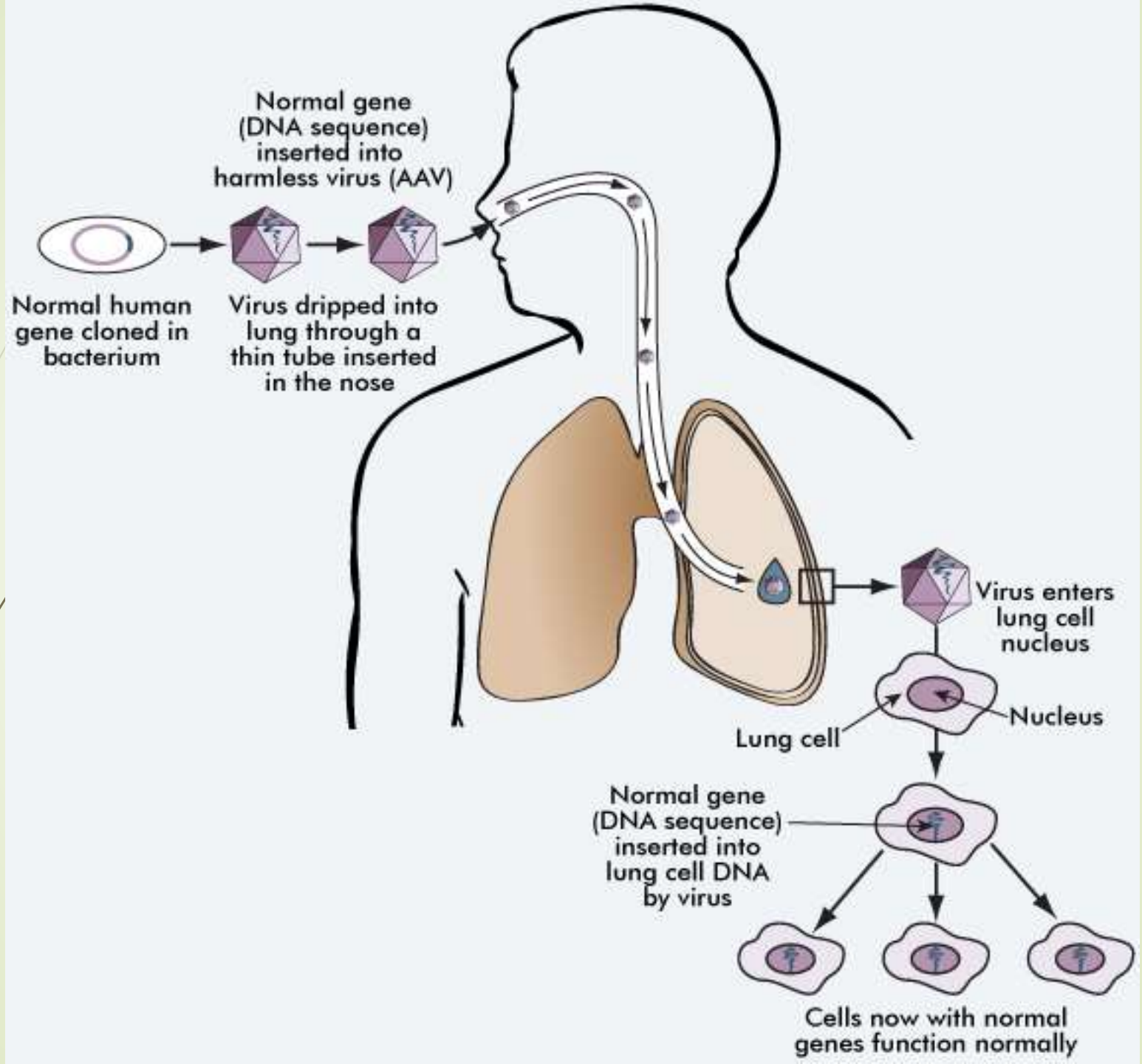
- It can be the only possible option in patients where individual cells cannot be cultured in vitro in sufficient numbers (e.g. brain cells).
- In vivo gene transfer is necessary when cultured cells cannot be re-implanted in patients effectively.



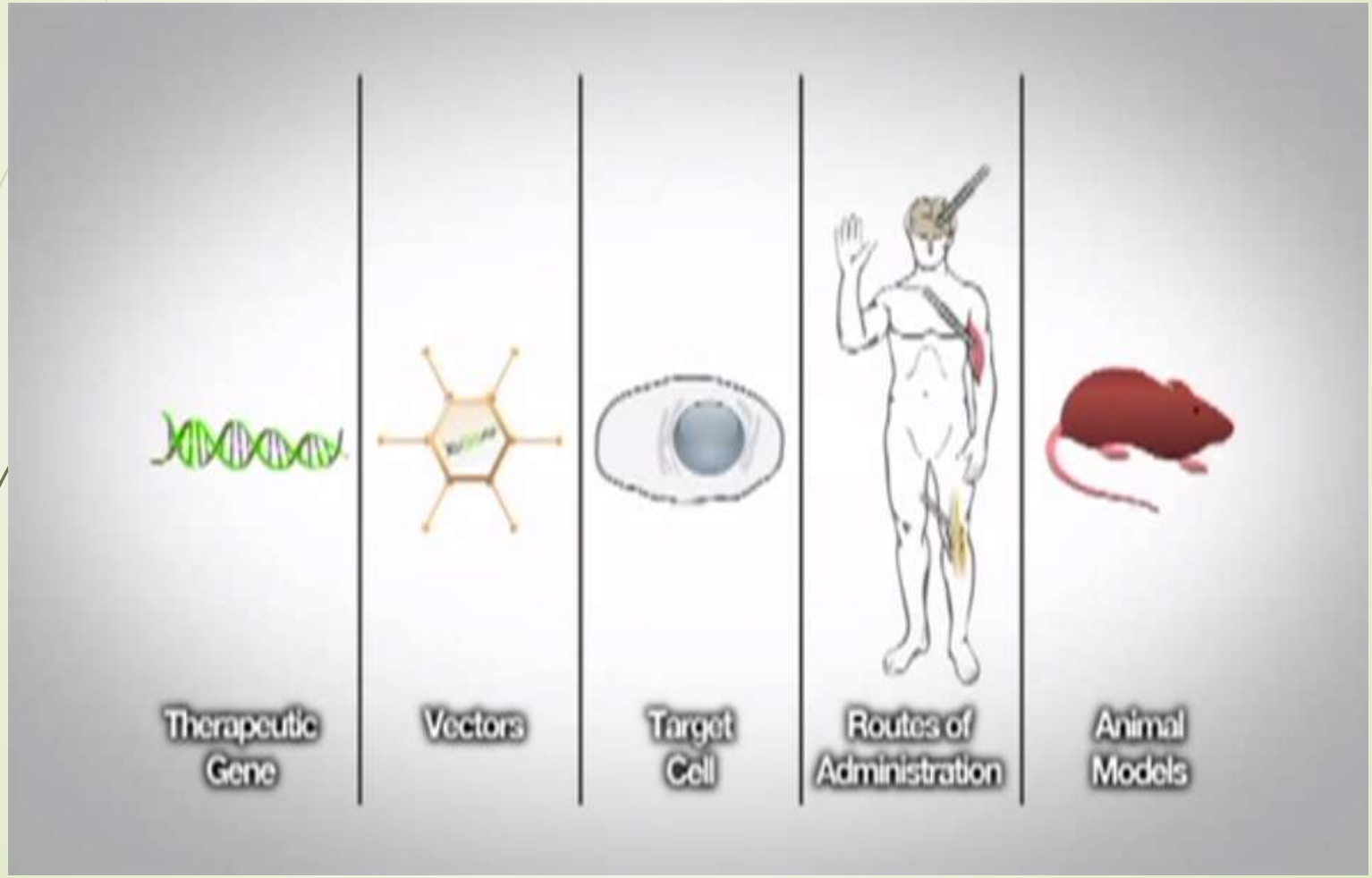
EXAMPLE OF IN VIVO GENE THERAPY

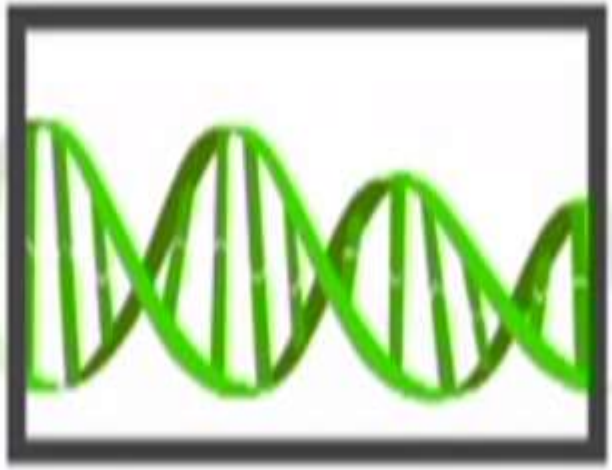
- In patients with cystic fibrosis, a protein called **cystic fibrosis trans-membrane regulator (CFTR)** is absent due to a gene defect.
- In the absence of CFTR chloride ions concentrate within the cells and it draws water from surrounding.
- This leads to the accumulation of sticky mucous in respiratory tract and lungs.
- Treated by in vivo replacement of defective gene by **adenovirus vector** .


Mutant CFTR Channel
does not move chloride ions, causing sticky mucus to build up on the outside of the cell
Outside cell
Mucus
Inside cell
Chloride Ions
Normal CFTR Channel
moves chloride ions to the outside of the cell


Normal gene
(DNA sequence)
inserted into
harmless virus (AAV)
Normal human
gene cloned in
bacterium
Virus dripped into
lung through a
thin tube inserted
in the nose
Virus enters
lung cell
nucleus
Nucleus
Lung cell
Normal gene
(DNA sequence)
inserted into
lung cell DNA
by virus
Cells now with normal
genes function normally

Key aspects


Therapeutic Gene
Vectors
Target Cell
Routes of Administration
Animal Models



Therapeutic Gene

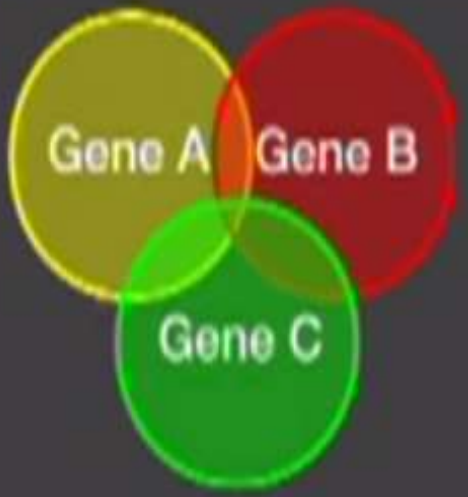
Monogenic diseases

- Monogenic diseases result from modifications in a single gene occurring in all cells of the body.
- A healthy gene will be introduced.
- Hemophilia
- Cystic fibrosis

Multifactorial diseases

Diabetes, Cancer, AIDS...

Interactions in more than one gene



Enviromental Factors
(Infections, Diet, Lack of exercise)

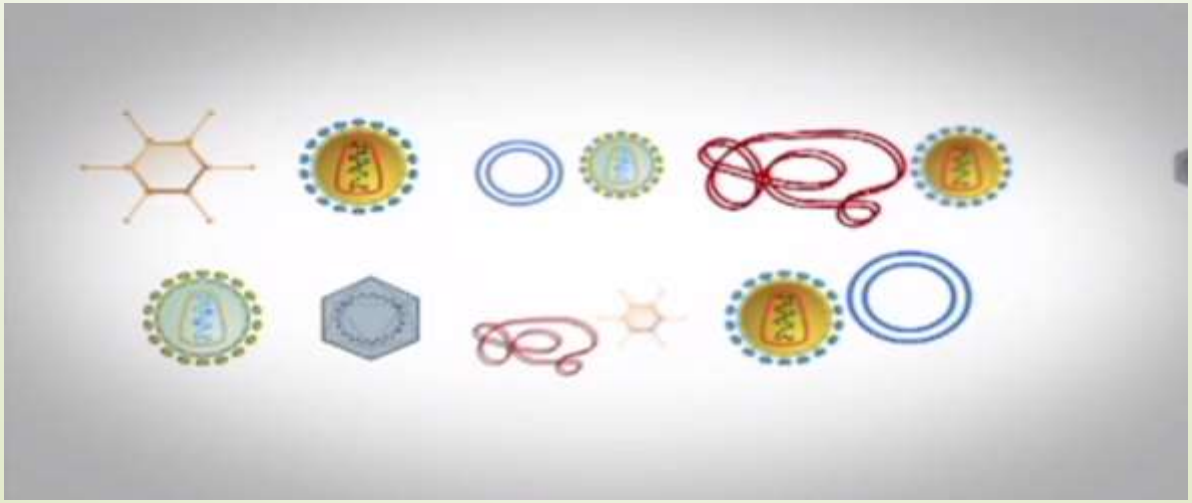
VECTORS IN GENE THERAPY

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

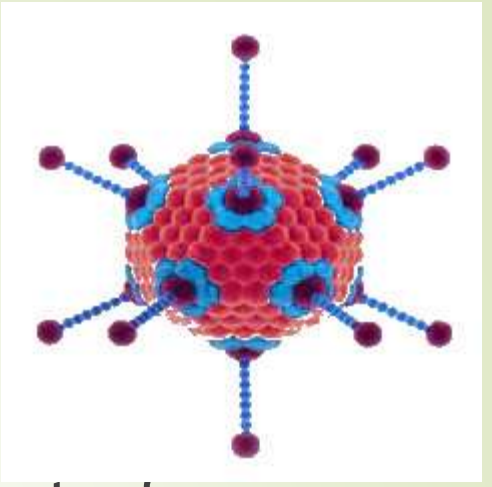
- 2 main classes
  - **Viral** vectors
  - **Non viral** vectors

Ideal vector

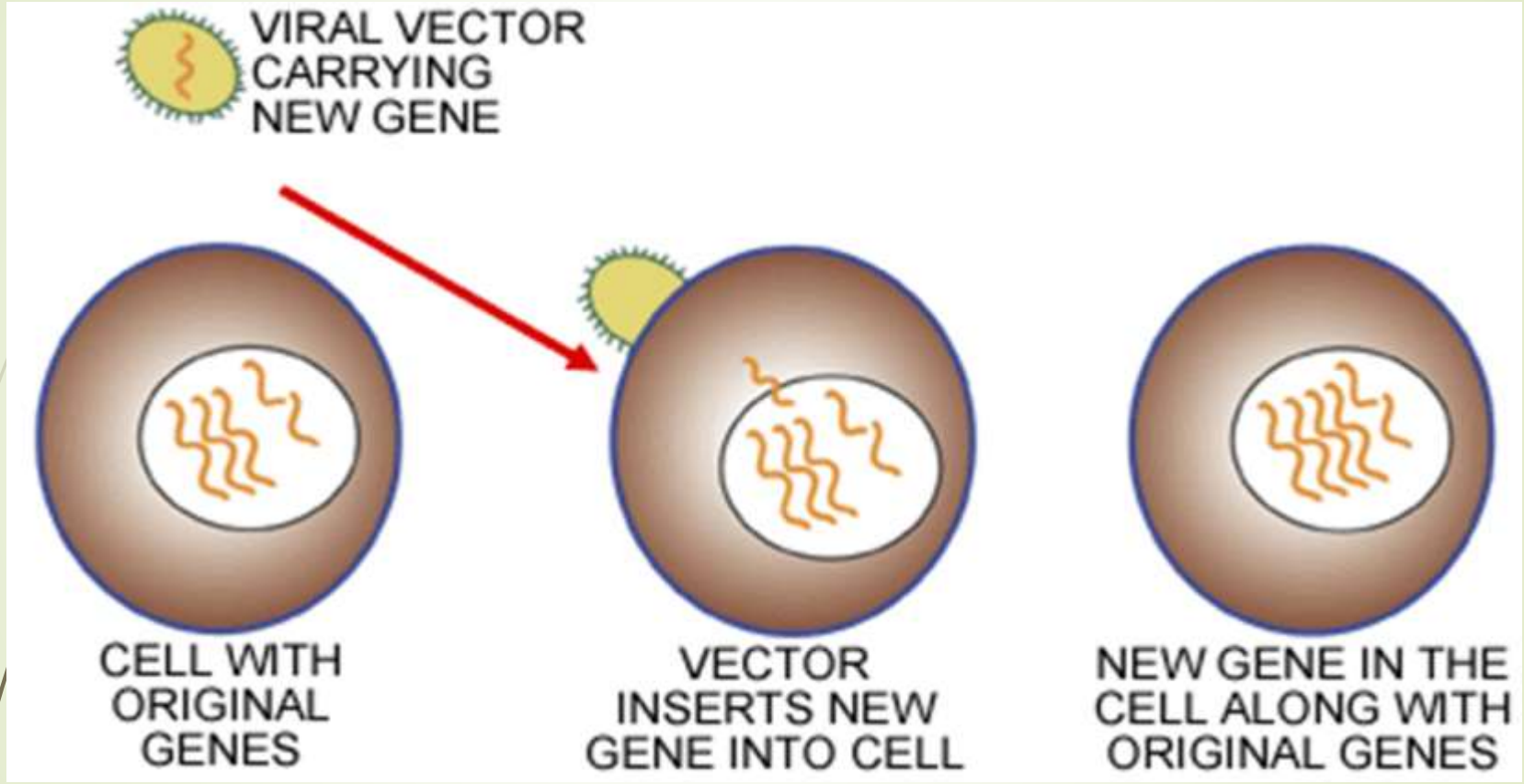
- TARGET the right cells
- INTEGRATE the gene in the cells.
- ACTIVATE the gene.
- AVOID harmful side effects.
- No universal vector exists.



VIRAL VECTORS



- Viruses introduce their genetic material into the host cell as part of their replication cycle.
- remove the viral DNA and using the virus as a vehicle to deliver the therapeutic DNA.
- The viruses used are altered to make them safe, although some risks still exist with gene therapy.


VIRAL VECTOR CARRYING NEW GENE
CELL WITH ORIGINAL GENES
VECTOR INSERTS NEW GENE INTO CELL
NEW GENE IN THE CELL ALONG WITH ORIGINAL GENES

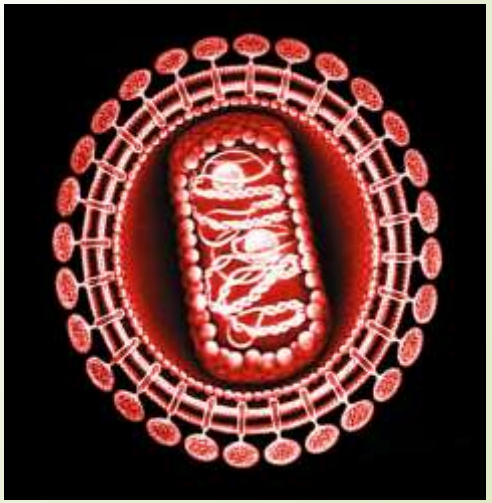
Types of viral vectors

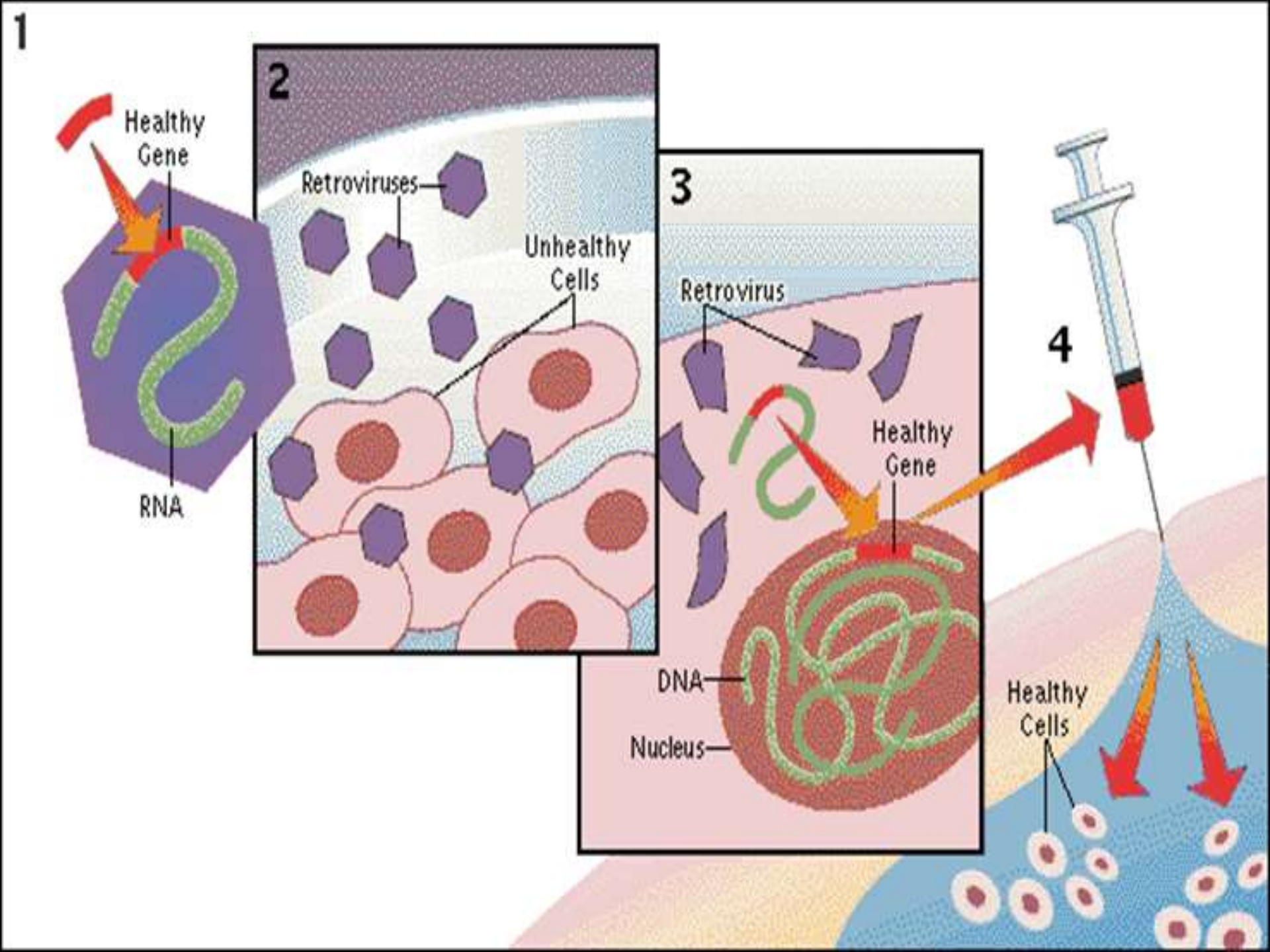
- A number of viruses have been used for human gene therapy, including :

1. Retrovirus
2. Adenovirus
3. Adeno-associated virus
4. Herpes simplex virus

1) RETROVIRUS VECTOR SYSTEM

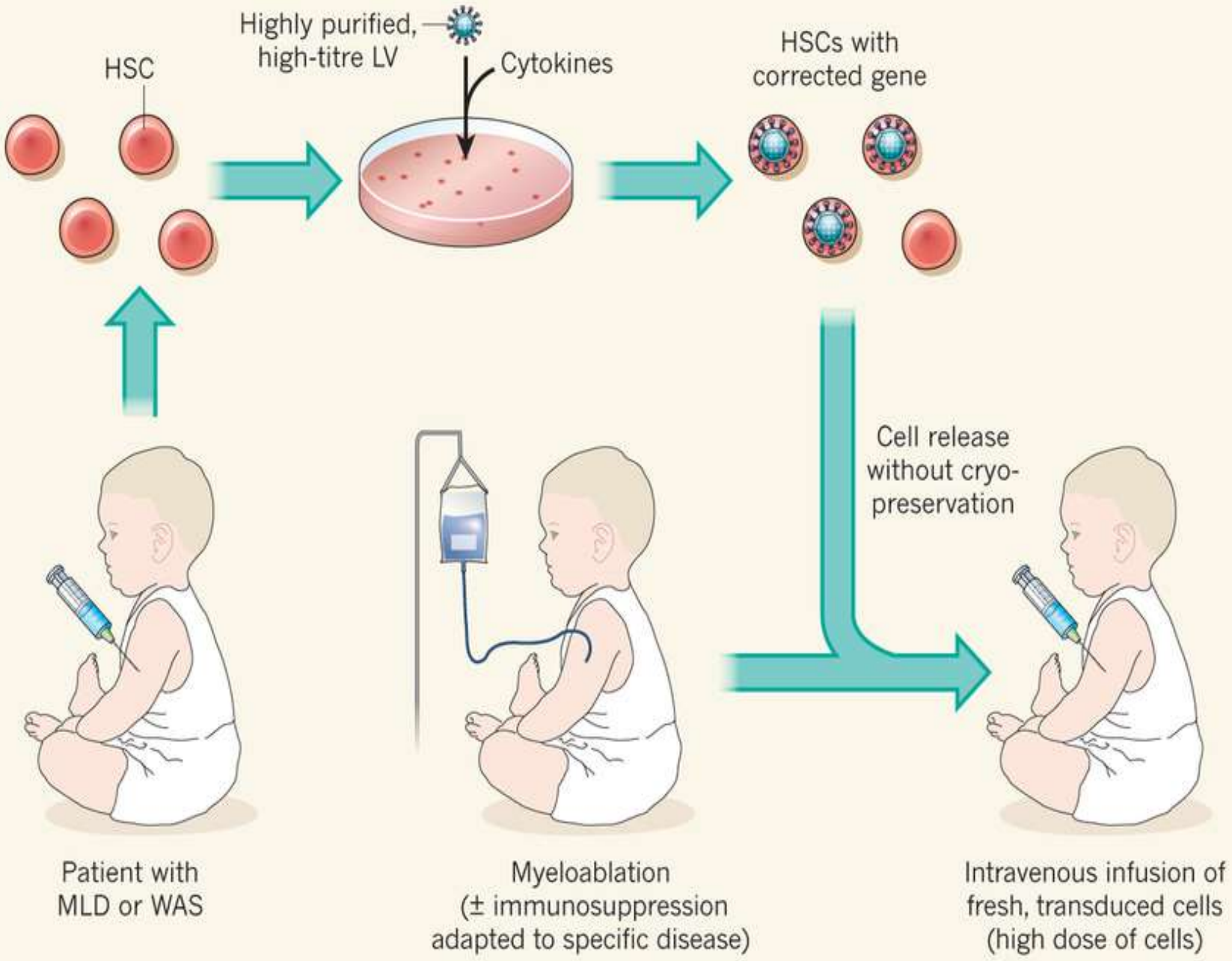
- The recombinant retroviruses have the ability to integrate into the host genome.
- Can carry a DNA of size – less than 3.4kb
- Target cell - dividing




1
Healthy
Gene
RNA
2
Retroviruses
Unhealthy
Cells
3
Retrovirus
Healthy
Gene
DNA
Nucleus
4
Healthy
Cells

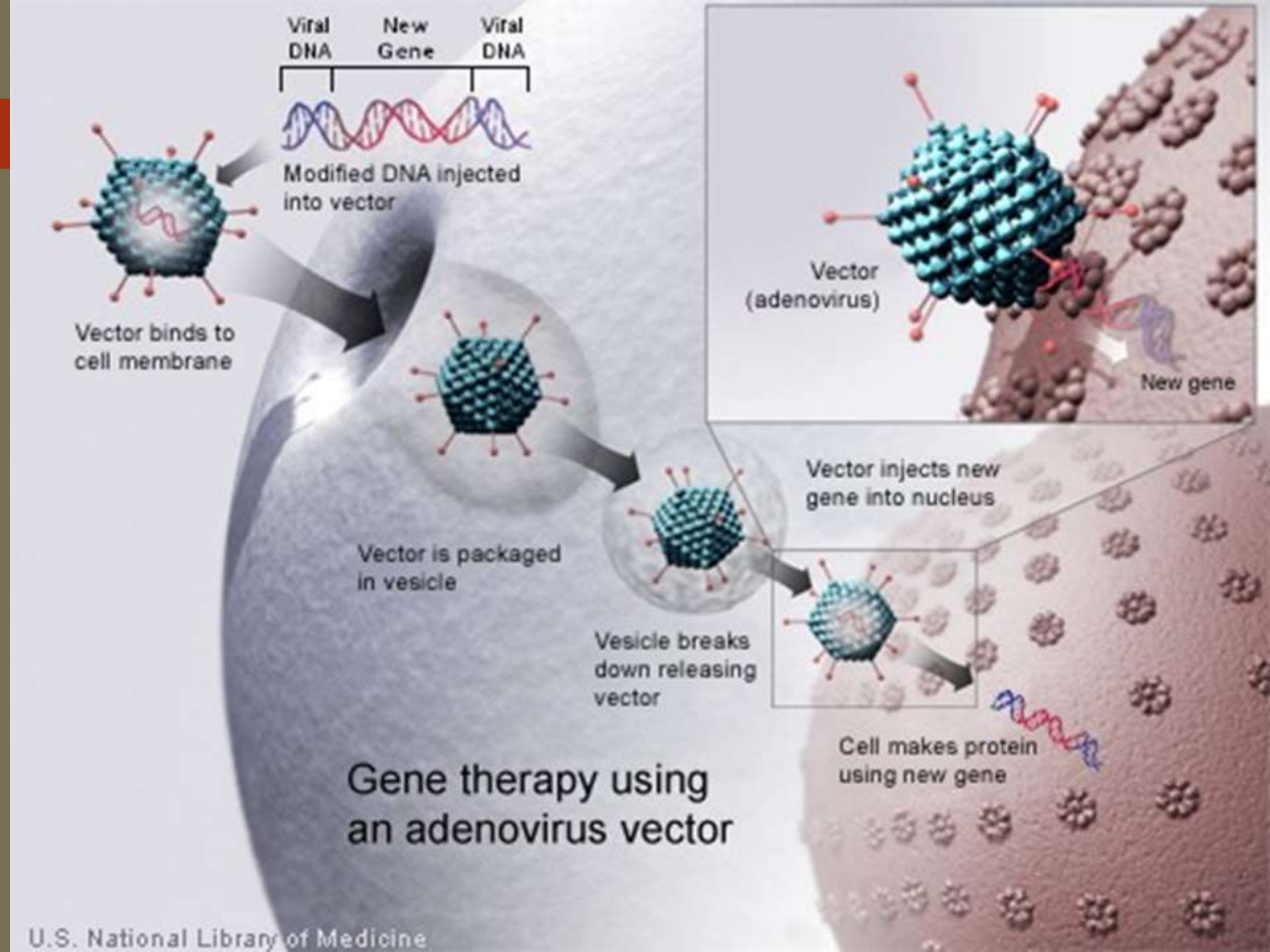
i. Lentivirus vector system:

- Subclass of Retroviruses.
- The viral genome in the form of RNA is reverse-transcribed when the virus enters the cell to produce DNA, which is then inserted into the genome at a random position via viral integrase enzyme.
- Target cells- dividing, non-dividing.


HSC
Highly purified, high-titre LV
Cytokines
HSCs with corrected gene
Cell release without cryo-preservation
Patient with MLD or WAS
Myeloablation (± immunosuppression adapted to specific disease)
Intravenous infusion of fresh, transduced cells (high dose of cells)

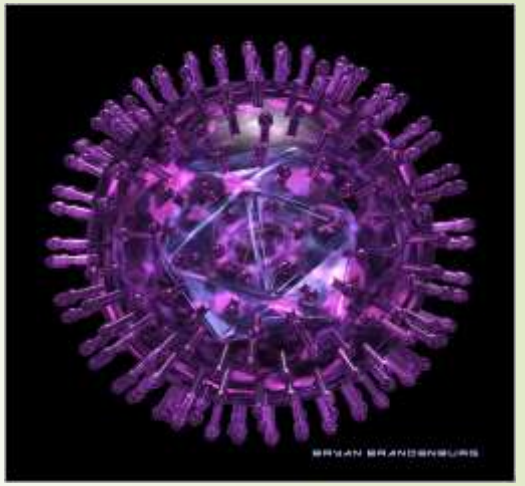
2) ADENO VIRUS VECTOR SYSTEM

- Adenoviral DNA does not integrate into the genome and is not replicated during cell division.
- Humans commonly come in contact with adenovirus, majority of patients have already developed neutralizing antibodies which can inactivate the virus.
- Target- **non dividing, dividing cells**.


Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector is packaged in vesicle
Vector injects new gene into nucleus
Vector (adenovirus)
New gene
Vesicle breaks down releasing vector
Cell makes protein using new gene
Gene therapy using an adenovirus vector
U.S. National Library of Medicine

3) ADENO ASSOCIATED VIRUS VECTOR

- It is a human virus.
- It is single stranded .
- AAV enters host cell, becomes double stranded and gets integrated into chromosome.
- AAV is not currently known to cause disease and consequently the virus causes a very mild immune response.
- Target- **non dividing, dividing cells**.



4)HERPEX SIMPLEX VIRUS VECTOR

- Viruses which have natural tendency to infect a particular type of cell.
- The Herpes simplex virus is a human neurotropic virus. This is mostly examined for gene transfer in the nervous system.

Table 1: Comparison of commonly used viral vectors in gene therapy

Virus	Gene material	Packaging capacity	Chromosome Integration	Key properties
Retrovirus	RNA	8 kb	Yes	Infects only dividing cells, persistent gene expression
Adenovirus	dsDNA	30 kb	No	Efficient short term gene expression
Adeno-associated virus	ssDNA	5 kb	No	Carry small amount of gene material
Lentivirus	RNA	8 kb	Yes	Infects both dividing and quiescent cells, persistent gene expression
Herpes simpex virus-1	dsDNA	40 kb	No	Strong tropism for neurons

Advantages

- Target specific types of cells.
- They're very good at targeting and entering cells.
- They can be modified so that they can't replicate and destroy cells.

Disadvantages

- They can cause immune responses in patients.
- They can carry a limited amount of genetic material. Therefore, some genes may be too big to fit into some viruses

NON VIRAL VECTOR SYSTEM

1. **PURE DNA CONSTRUCT**
   - Direct introduction of pure DNA construct into target tissue.
   - Efficiency of DNA uptake by cells and expression rather low.
   - Consequently, large quantities of DNA have to be injected periodically.

Cont....

2) DNA MOLECULAR CONJUGATES

- Commonly used synthetic conjugate is poly- L- lysine bound to specific target cell receptor.
- Therapeutic DNA is then made to combine with the conjugate to form a complex.
- It avoids lysosomal breakdown of DNA

3) LIPOPLEXES

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

4) HUMAN ARTIFICIAL CHROMOSOME

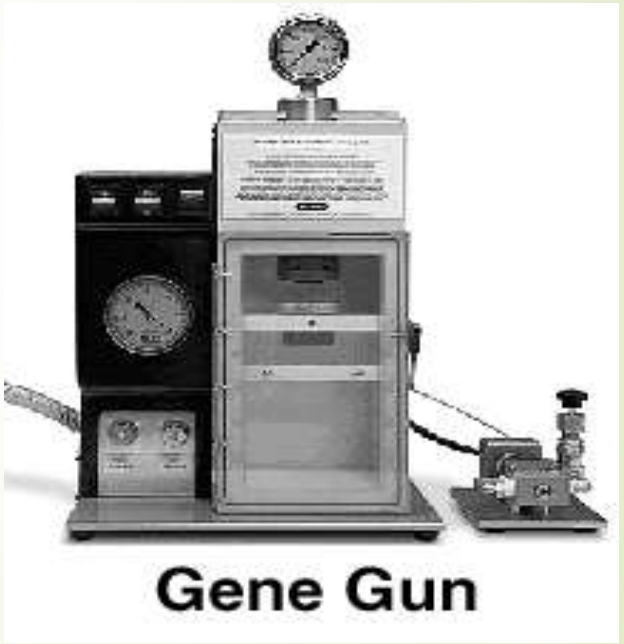
- Can carry a large DNA i.e., with one or more therapeutic genes.

METHODS OF GENE DELIVERY

PHYSICAL METHODS

1) Gene Gun

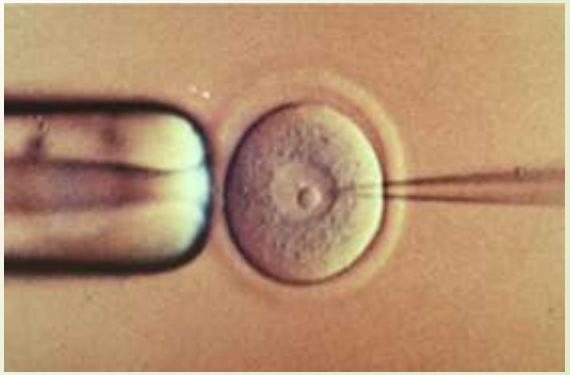
- Employs a high-pressure delivery system to shoot tissue with gold or tungsten particles that are coated with DNA



Gene Gun

Microinjection

- Process of using a glass micropipette to insert microscopic substances into a single living cell.
- Normally performed under a specialized optical microscope setup called a micromanipulator.



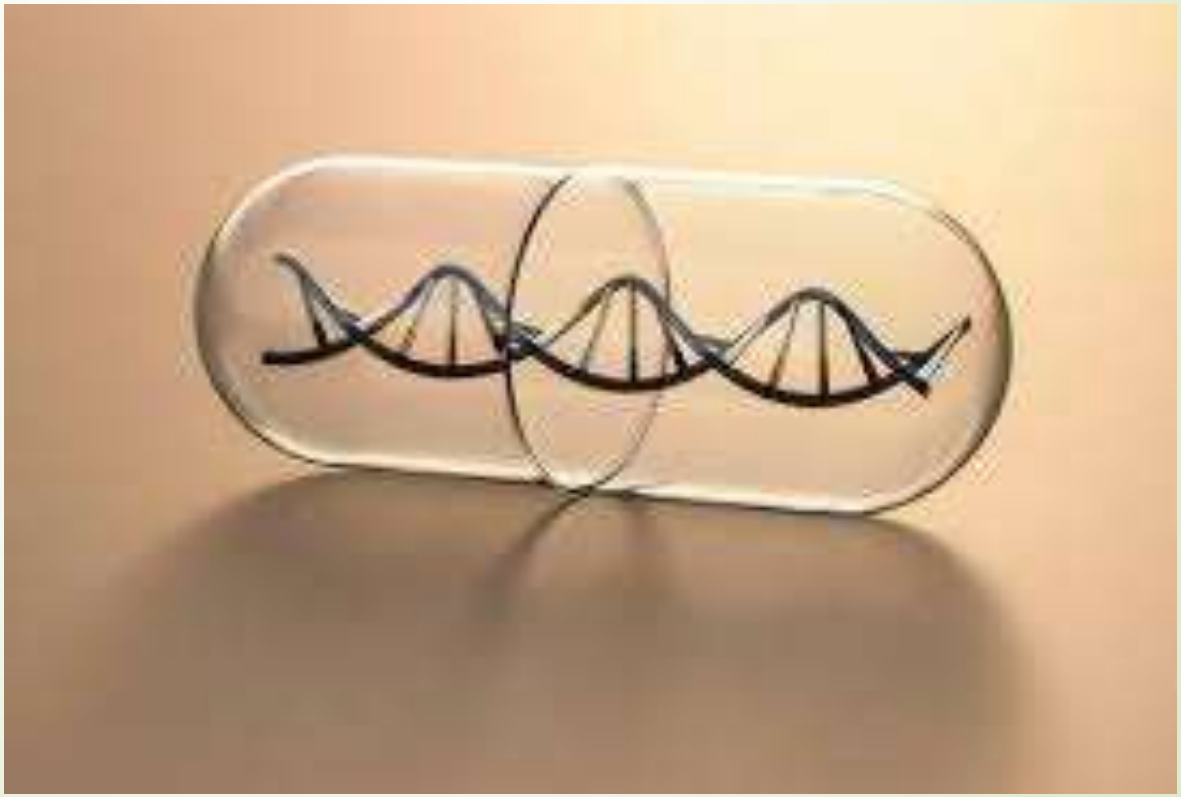
CHEMICAL METHODS

- **USING DETERGENT MIXTURES**
  - Certain charged chemical compounds like Calcium phosphates are mixed with functional cDNA of desired function.
  - The mixture is introduced near the vicinity of recipient cells.
  - The chemicals disturbs the cell membrane, widens the pore size and allows cDNA to pass through the cell.

LIPOFECTION

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

SUCCESS CASES OF GENE THERAPY



GENE THERAPY CURES BLINDNESS

- Cure blindness of inherited condition
- Leber's conginetal amaurosis

- inherited disease caused by an abnormality in a gene called RPE65.

- The condition appears at birth or in the first few months of life and causes progressive worse and loss of vision.

HOW IT WORKS??

- used harmless viruses
- enable access to the cells beneath the retina of patients
- By using a very fine needle

-safe in an extremely fragile tissue and can improve vision in a condition previously considered wholly untreatable.

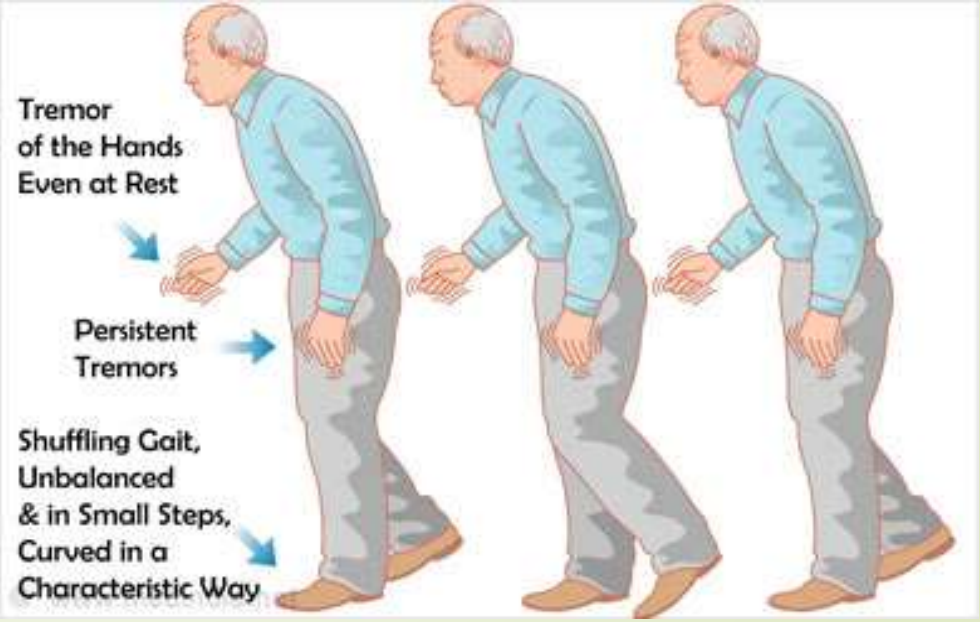
GENE THERAPY REDUCES PARKINSON'S DISEASE SYMPTOMS

- It has significantly improved the weakness of the symptoms such as tremors, motor skill problems, and rigidity
- Done with local

anesthesia, used

a harmless, inactive

virus [AAV-2]



ADVANTAGES

- Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis, ADA- SCID etc.
- 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.

DISADVATAGES

- Long lasting therapy is not achieved by gene therapy; Due to rapid dividing of cells benefits of gene therapy is short lived.
- Immune response to the transferred gene stimulates a potential risk to gene therapy.

- Disorders caused by defects in multiple genes cannot be treated effectively using gene therapy.
- Viruses used as vectors for gene transfer may cause toxicity, immune responses, and inflammatory reactions in the host.

ETHICAL ISSUES

- Who will have access to therapy?
- Is it interfering with God's plan?
- Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence etc.?
- Is it alright to use the therapy in the prenatal stage of development in babies?

CONCLUSION

- Theoretically, gene therapy is the permanent solution for genetic diseases.
- But it has several complexities. At its current stage, it is not accessible to most people due to its huge cost.
- A breakthrough may come anytime and a day may come when almost every disease will have a gene therapy
- Gene therapy have the potential to revolutionize the practice of medicine.


THANK YOU