

Tutorial 1 : Gene therapy

I. Introduction

Gene therapy is an innovative therapeutic approach that aims to treat genetic disorders by introducing, removing, or modifying genetic material inside patient cells. Its fundamental objective is to restore a defective cellular function by delivering a functional genetic sequence capable of producing a therapeutic molecule. Because naked nucleic acids are unstable and cannot efficiently enter target cells, gene delivery requires the use of specialized systems called vectors. Among these systems, viral vectors are the most widely studied and used because viruses naturally possess mechanisms allowing them to enter cells and deliver their genetic material.

II. Viral vector design:

It involves modifying a natural virus to create a safe vehicle for delivering a therapeutic gene.

1. Virus disarming: A natural parental virus is modified by removing genes responsible for pathogenicity, making it safe for therapeutic use.
2. Insertion of the transgene: it is inserted into an expression cassette containing a promoter.
3. Helper system: Viral genes required for replication and structural proteins (capsid, enzymes) are provided separately by a helper construct. These genes are only used to produce viral particles and are not included in the final therapeutic vector.
4. Production in packaging cells: The vector genome and helper construct are introduced into packaging cells. The cells produce viral particles using the helper genes and package the therapeutic vector genome inside the viral capsid.
5. Final product: A therapeutic viral vector capable of delivering the transgene to target cells without causing disease.

III. Main viral vectors used in gene therapy

- *Lentiviral and Retroviral Vectors (LV/RV)*

Their RNA genome is converted into DNA by reverse transcriptase, allowing integration into the host genome and stable, long-term gene expression.

Advantages: Long-term gene expression and stable genetic modification.

Limitations: Risk of insertional mutagenesis due to genomic integration.

Applications: Hematopoietic stem cell gene therapy and CAR-T cell therapies.

- *Adenoviral Vectors (Ad)*

Adenoviruses are non-enveloped, dsDNA viruses capable of efficiently transducing dividing and non-dividing cells. Their genetic material generally remains outside the host chromosomes.

Advantages: High gene delivery capacity and strong gene expression.

Limitations: Potentially strong immune responses and usually transient gene expression.

Applications: Cancer gene therapy and therapeutic gene delivery.

- *Adeno-Associated Viral Vectors (AAV)*

AAVs are small, non-enveloped, single-stranded DNA viruses widely used in gene therapy.

Advantages: Relatively low immunogenicity and sustained gene expression.

Limitations: Small packaging capacity (approximately 4.7 kb) and possible immune responses.

Applications: Retinal, neuromuscular, and certain neurological genetic disorders.

- *Herpes Simplex Virus Vectors (HSV)*

Large, enveloped, dsDNA vectors with a high capacity for carrying therapeutic genes.

Advantages: Very large genetic capacity and neuronal tropism.

Limitations: Complex vector engineering and immune responses.

Applications: Neurological disorders and cancer gene therapy.

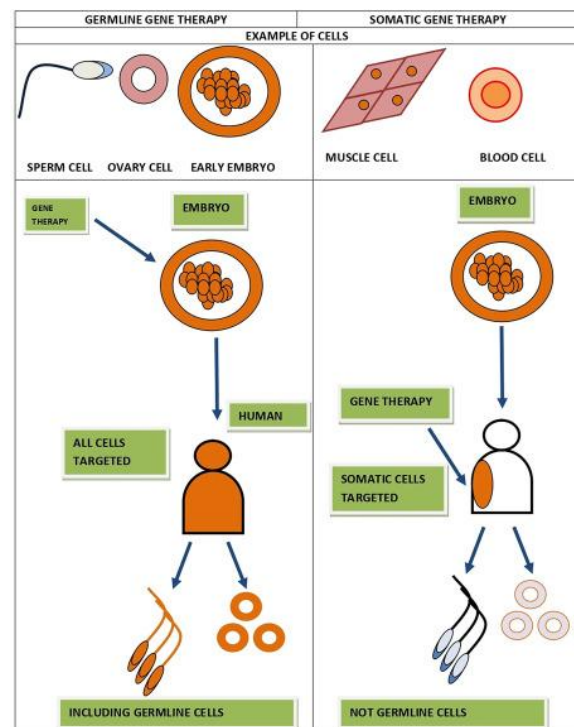
IV. Germline and somatic gene therapy

1. Germline Gene Therapy

Germline gene therapy involves modifying the genetic material of embryos or reproductive cells (sperm or eggs). When performed at an early embryonic stage, the modification may be present in all cells of the developing individual, including germ cells. Therefore, genetic changes can be transmitted to future generations. However, this approach raises major ethical and safety concerns and is not currently accepted for clinical reproductive use.

2. Somatic Gene Therapy

Somatic gene therapy involves modifying non-reproductive cells to treat or prevent genetic diseases. Unlike germline therapy, only specific cells or tissues are targeted, such as blood, liver, or muscle cells. These genetic modifications affect only the treated individual and are not inherited by future generations. Somatic gene therapy is the approach used in current clinical gene therapy treatments.



V. Strategies of gene therapy

V.1. In vivo gene therapy : it involves the direct administration of a viral vector carrying a therapeutic gene into the patient's body. Genetic modification occurs directly within the target cells.

Main steps:

1. Vector administration: The therapeutic viral vector is injected into the patient.
2. Target cell recognition: The vector reaches the target tissue and interacts with specific cellular receptors.
3. Gene delivery: The vector enters the target cells and delivers the therapeutic gene.
4. Gene expression: The introduced gene is expressed through transcription and translation, producing a functional protein that helps correct the cellular defect.

The effectiveness of this approach depends largely on viral tropism, which refers to the preference of a viral vector for certain tissues or cell types.

Advantages: Direct treatment of tissues, including difficult-to-access organs such as the brain, retina, and muscles, without removing patient cells.

Limitations: Possible immune responses against the viral vector, neutralizing antibodies, and difficulties in precisely targeting specific cells.

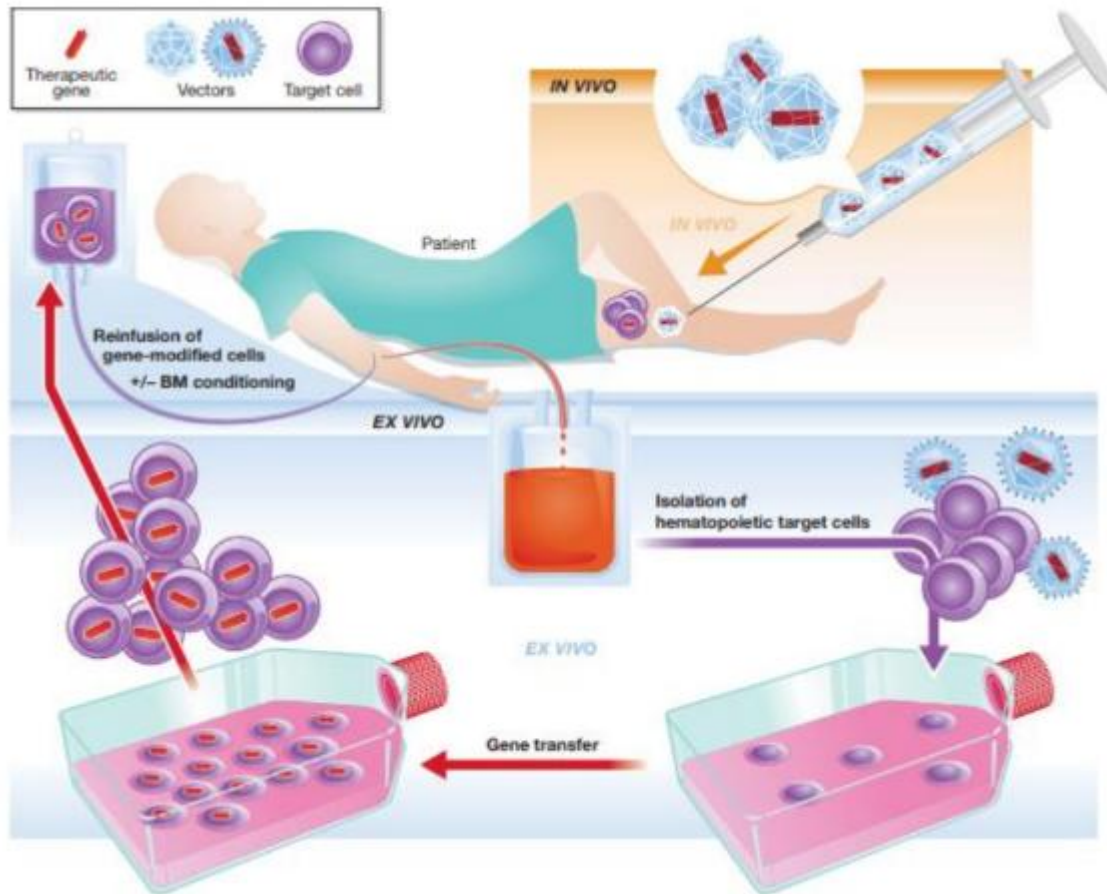
V.2. Ex Vivo Gene Therapy: it involves genetically modifying the patient's cells outside the body before reintroducing them.

Main steps:

1. Cell extraction: Specific cells, such as hematopoietic stem cells or T lymphocytes, are collected from the patient.
2. Genetic modification: A viral vector introduces the therapeutic gene into the extracted cells.
3. Cell expansion: Modified cells are cultured to obtain sufficient numbers for treatment, when necessary.
4. Quality control: The modified cells are tested to verify their genetic modification, functionality, and safety.
5. Reinfusion: The genetically modified cells are administered back to the patient to restore or improve the defective function.

Advantages: Greater control over genetic modification, allowing cells to be tested before administration.

Limitations: Complex, costly laboratory procedures and applicability mainly to cells that can be collected, modified, and reintroduced.



In Vivo and Ex Vivo gene therapy