- **What is a gene?**
  - A sequence of chromosomal DNA that is required for production of a functional protein (it contains both introns and exons).

- **What is gene therapy?**
  - It is the treatment of human diseases by transferring genetic material into a person’s cell to fight/prevent the disease.

- **Types of gene therapy:**
  - There are two types of gene therapy:
    - **Germ-line Gene Therapy**
      - Targeting gonads, sperm and ova
    - **Somatic Cells Gene Therapy**
      - Ethically unacceptable
      - Transmitted to offspring
      - Targeting cells, tissues and organs in which the disorder is manifested

- **Is gene therapy applied in all diseases?** → no, there are requirements:
  - **Disease:**
    - The disease must be serious.
    - Incurable with conventional treatment.
    - Chronic (requires life-long treatment).
  - **Gene:**
    - The gene responsible for the disease must be identified.
    - Copies of the gene can be made in the lab.
    - Role of protein encoded by the gene is known.
  - **Target organ:**
    - Readily accessible.
    - Long survival time.
    - Ability to replicate itself.
  - **Ethical approval.**

- **Diseases which are targeted by gene therapy:**
  - **Inherited disease with single-gene defect:**
    - Severe Combined Immunodeficiency disease (SCID).
    - Duchenne muscular dystrophy.
    - Hemophilia.
    - β-thalassemia.
    - Cystic fibrosis.
  - **Poly-genic (multiple gene-defects are responsible for the disease) or non-inherited diseases:**
    - Infectious diseases: HIV and hepatitis-C
    - Rheumatoid Arthritis (RA).
    - Cancer (it is the most targeted disease in gene therapy).
    - Cardiovascular diseases.

- **Gene therapy – step by step:**
  - Identify the gene which is responsible for the disease.
  - Make copies of the normal gene.
• Insert the copies into a vector (mostly virus):
  ✓ Remove the viral genome and then insert the gene of interest.
• Infect affected cells with the vector:
  ✓ Normal gene is carried into the nucleus.
  ✓ The DNA may integrate into the genome.
• Activate the gene (transcription & translation take place).
  - There are two approaches of gene delivery:
    • In vivo approach: injection of the vector into the body and specifically target affected cells.
    • Ex vivo approach: delivering the gene to cells while they are outside the body.
• Strategy of gene therapy:
  • Loss of function mutation: insert a copy of normal gene.
  • Gain of function mutation: replace with the normal gene.
• Cancer gene therapy:
  ✓ Stimulation of natural killing of tumor cells (IL-2).
  ✓ Anti-angiogenic genes (inhibiting VEGF).
  ✓ Supply tumor suppressor genes (p53).
  ✓ Inhibition of oncogenic proteins (such as bcr-abl).
  ✓ Transporting anti-cancer genes selectively into cancer cells using nanotechnology (magic bullet!).
• What are the characteristics of an ideal vector?
  • Has an adequate carrying capacity.
  • Easy to produce in high concentrations.
  • Targeting specific tissues.
  • Stable.
  • No immune response, non-inflammatory and non-toxic.
  • High efficiency.
  • Long duration of expression.
• Methods of gene delivery:
  • Transduction: viral-mediated gene transfer.
  • Transfection: non-viral mediated gene transfer.
• Types of vectors:
  • Viral vectors (transduction):
    ✓ Retroviruses: these are RNA viruses encoding for reverse transcriptase enzyme. They are used in ex vivo gene therapy.
      ✓ Advantages:
        ➢ DNA integrated into host genome.
        ➢ Non-immunogenic and non-toxic.
      ✓ Disadvantages:
        ➢ Small insert size.
        ➢ Infect only dividing cells.
        ➢ Risk of insertional mutagenesis which leads to activation of oncogenes.
    ✓ Lentiviruses:
      ✓ Advantages:
        ➢ Infect dividing and non-dividing cells.
        ➢ Easier to culture than retroviruses.
      ✓ Disadvantages:
        ➢ Risk of insertional mutagenesis which leads to activation of oncogenes.
- **Adenoviruses (these are DNA viruses):**
  - **Advantages:**
    - No risk of insertional mutagenesis.
    - Large.
    - Infect dividing and non-dividing cells.
  - **Disadvantages:**
    - Short duration of expression.
    - Immunogenic with high toxicity (might lead to death).

- **Adeno-associated viruses:**
  - **Advantages:**
    - Inserted into chromosome 19.
    - Infect wide variety of cells.
    - Long duration of expression.
    - Low immune response.
  - **Disadvantages:**
    - Carry small insert size.

- **Herpes virus:**
  - **Advantages:**
    - Infects nervous tissue.
  - **Disadvantages:**
    - Neurotoxicity.

- **Non-viral vectors (transfection):**
  - **Types:**
    - Liposomes.
    - Direct injection of naked DNA.
    - Receptor-mediated endocytosis.
    - Oligonucleotides.
  - **Advantages:**
    - Carrying large insert size.
    - Targeting specific cells.
    - Non-immunogenic.
    - No risk of insertional mutagenesis.
  - **Disadvantages:**
    - Low efficiency.
    - Transient expression (short-term expression).
    - Degradation of protein-DNA conjugate (by lysosomes of the cell).

- **What are the problems of gene therapy?**
  - Short-lived nature of gene therapy.
  - Immune response to vector.
  - General toxicity of viral vectors.
  - Insertional mutagenesis.
  - Multi-gene disorders are hard to treat because you need to introduce more than one gene.
  - Contamination of germ-line cell.
  - Expensive.

- **Current status of gene therapy:** mostly experimental and has not proven very successful in clinical trials.

- **Gene doping:** non-therapeutic use of cells, genes, genetic elements of or the modulation of gene expression having the capacity to improve athletic performance.