

# Application Syllabus

## Gene Therapy – Summary

### Learning Outcomes:

- Describe two types of genetic disease. E.g. SCID, cystic fibrosis, using viral and non-viral gene delivery systems, that can be treated with gene therapy.
- Explain the factors that keep gene therapy from becoming an effective treatment for genetic diseases.
- Discuss the social and ethical considerations for the use of gene therapy.

### Definition:

- Adding of genetic material so that a genetic defect or genetic disease can be corrected (most commonly by introducing a normal, healthy allele).
- Only feasible if due to defects in single genes.

### Types:

- Somatic
- Gametic

### Benefits

- Offer alternative treatment if conventional methods are ineffective
- Potentially permanent (germline)
- Effective within a lifetime (somatic)

### Guidelines:

- Gene to be transferred must be available.
- An effective method of introducing the gene into human cells is necessary.
- The target tissue or target cells must be accessible to the gene transfer technique.
- The gene therapy must not harm the patient.
- There must be no other form of effective therapy available.

### 4 Strategies:

- Only need to know Gene-augmentation therapy (GAT)
  - Normal allele may be inserted into a non-specific location within the genome to overcome the effects of the non-functional allele.

### Ex Vivo

1. Cells removed from body
  2. Cells cultured in vitro for a period of time to increase cell number
  3. Normal copies of gene transferred in vitro into cells
  4. Genetically correct cells returned to patient
- Works well with blood disorders
  - Cells must be amenable to culture
  - No immune response (patient's own cells)

## In Vivo

- Transfer of genes directly into cells of the body

## Vectors

- Ideal attributes:
  - High efficiency of uptake
  - Minimal intra cellular degradation while transporting therapeutic gene
  - Sustained expression of therapeutic gene at a sufficient level
- Viral-mediated
  - Viral genome manipulated – disease causing genes are removed/inactivated
  - Therapeutic genes inserted
  - Virus' ability to replicate must be destroyed
    - No new viral particles are created
    - Host does not get lysed.
- Non-viral mediated

Gene delivery system	Advantages	Disadvantages
Retrovirus <ul style="list-style-type: none"> <li>• HIV</li> </ul>	<ul style="list-style-type: none"> <li>• Most established and efficient method</li> <li>• Able to integrate into the DNA of human cells</li> <li>• Can accommodate more foreign DNA than most DNA and RNA viruses</li> </ul>	<ul style="list-style-type: none"> <li>• Can only carry small pieces of DNA (&lt;8kb)</li> <li>• Host cell must be rapidly dividing for viral DNA to be incorporated into chromosome, but most adult cells have fully differentiated and stopped dividing</li> <li>• Integrase inserts viral genome randomly. Might cause insertional mutagenesis → cancer</li> </ul>
Adenovirus <ul style="list-style-type: none"> <li>• Common cold</li> </ul>	<ul style="list-style-type: none"> <li>• Able to infect both dividing and non-dividing cells effectively</li> <li>• Specific cell targeting possible by engineering viral surface proteins</li> <li>• No integration of DNA (no cancer)</li> <li>• Larger genome (&gt;30kb of non viral DNA)</li> </ul>	<ul style="list-style-type: none"> <li>• Will not integrate into the host cell's genome</li> <li>• Descendants of cell will not have gene</li> <li>• Adenoviruses can trigger immune response in patient. (Viral surface proteins then have to be removed.)</li> </ul>
Adeno-associated virus <ul style="list-style-type: none"> <li>• Insert on specific site on chromosome 19</li> </ul>	<ul style="list-style-type: none"> <li>• Will integrate into host cell 95% of time.</li> <li>• Non pathogenic (not cause disease)</li> <li>• No immune response</li> <li>• Proteins on viral surface can be engineered to ensure specific targeting</li> <li>• Specific insertion site ensures no disruption of other genes</li> </ul>	<ul style="list-style-type: none"> <li>• Small amount of DNA. (&lt;5kb)</li> </ul>
Liposomes	<ul style="list-style-type: none"> <li>• Can carry large amounts of DNA (no limit)</li> </ul>	<ul style="list-style-type: none"> <li>• Low transfection efficiency</li> <li>• Unless specifically engineered to do so,</li> </ul>

	<ul style="list-style-type: none"> <li>• Can be targeted to specific cells/tissues by modification of liposome membrane</li> <li>• No immune response</li> <li>• No replication of virus</li> </ul>	<p>DNA will not integrate into host cell's genome.</p> <ul style="list-style-type: none"> <li>• Low integration efficiency.</li> </ul>
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## SCID (Severe Combined Immuno-Deficiency)

### X-Linked

- Mutation in the gene (X-chromosome) producing interleukin gamma chains.
- This disrupts the interleukin signalling and therefore prevents formation of T and B Lymphocytes
- Therefore compromising the immune system of the body

### Adenosine Deaminase (ADA) deficiency

- Mutation causes defective ADA enzyme
  - ADA breaks down purines
- Defective ADA enzyme → deoxyATP accumulates
- High levels of deoxyATP is toxic to T and B Lymphocytes.

### Conventional Treatment

- X-Linked
  - Stem cell transplant
- ADA
  - Enzyme replacement therapy
    - Weekly injections of ADA
- Transfection: viral carriers
- Lipofaction: liposomes

### Genetic Treatment

- X-Linked
  - Same as ADA treatment below, just insert the gamma chain gene to produce functional interleukins.
- ADA
  - Genetically disable a retrovirus
  - Insert normal ADA gene into retrovirus
  - Extract T lymphocytes and allow modified retroviruses to infect them
    - Therefore integrating ADA gene into T lymphocytes
  - T lymphocytes cultured, to ensure ADA gene is active

- T lymphocytes reimplanted into cell

## **Cystic Fibrosis**

- 3bp deletion on chromosome 7
- results in loss of phenylalanine
- therefore Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is missing
- $\text{Cl}^-$  and  $\text{Na}^+$  transport in lung epithelium disrupted, building up thick mucous in the lungs
  - Water retained in cells owing high concentration of salts inside the epithelium.

## Treatment

- Isolated CFTR gene is spliced into plasmid vector
- Package plasmid vector into liposome and deliver to lungs via nasal spray.
- Liposomes fuse with epithelial cell membrane and the plasmid is introduced into the cells.
  - Via lipofaction

## OR

- Adeno-virus transfection is also possible.