Gene Therapy

Definitions
Transfect = Introduction of foreign DNA into eukaryotic cells using non-viral methods
Transduce = Introduction of foreign DNA into cells using viral vectors
Transform = Direct uptake of genetic material from surroundings

Gene Therapy

- Introduction of normal, functional allele into cells with defective mutant alleles so that cells can produce a functional protein
- Treats single gene recessive disorders where affected individuals have 2 copies of the recessive allele in their genome

Targets

- Somatic cells → Treatment effective for patient but not heritable
- Germline cells → Gamete/fertilised egg treated ⇒ Normal functional allele present in all cells from modified embryo ⇒ Treatment effective for offspring and subsequent generations but not for the patient

Approaches

- **Ex vivo**: Cells removed from body → Modified outside body → Modified cells transplanted back into body
- **In vivo**: Cells modified while still in the body

Method

- Normal functional allele isolated and linked to appropriate promoter
- Inserted into vector (e.g. virus/liposome) OR Direct gene transfer into the cell (e.g. microinjection/ electroporation)
- Introduced into target cell that needs correcting
- Introduced allele expresses the protein that is lacking

Severe Combined Immunodeficiency (SCID)
Description:

- Heritable immunodeficiency disease where T and B lymphocytes are reduced in numbers or malfunctioning
- Types:
    - X-linked SCID
        - X-linked recessive

        - Mutations in gene coding for **common gamma chain** (cell receptor for interleukins - proteins that stimulate T & B lymphocytes) in X chromosome
        - No functional interleukin receptors on T & B lymphocytes ⇒ T & B lymphocytes fail to proliferate and differentiate
    - Adenosine deaminase (ADA) enzyme deficient SCID
        - Autosomal recessive
        - Mutations in gene coding for **ADA** on chromosome 20
        - No ADA to break down adenosine ⇒ Accumulation of toxic deoxyadenosine ⇒ T & B lymphocytes die

Conventional Treatment:

- Stem cell transplant ⇒ New stem cells can rebuild immune system
    - Limitations: Patient immune system may reject introduced cells
- Weekly ADA replacement therapy ⇒ Hinders build up of dATP so that B & T lymphocytes can mature
    - Limitations: Requires constant treatment + Efficacy of treatment varies in different patients

Gene Therapy:

- **Ex-vivo**
- Viral mediated gene delivery system → **Retroviruses**
    - e.g. Lentivirus with genes responsible for viral replication removed
- Steps:
    1. T cells removed from patient
    2. Insert **normal, functional ADA allele** (or common gamma chain allele) into T lymphocytes using retroviral vector
    3. Transplant cells back into patient
    4. Recombinant T lymphocytes will produce ADA/functional interleukin receptor
- Advantages of **viral** mediated system
    - Specific glycoproteins enables virus to target specific cell types (specificity)
    - High transduction efficiency as viruses possess a mechanism to attach to and enter specific target cells
    - Retrovirus uses **integrase** to integrate normal functional allele into host cell genome, providing stable long term gene expression
    - Greater chance of delivering functional allele into nucleus via nuclear pores
- Disadvantages of **viral** mediated system
    - Capsid of virus poses a limit on size of normal functional allele that can be delivered to target cells
    - Random insertion of normal functional allele into genome may lead to **insertional mutagenesis** whereby tumour suppressor genes are inactivated and/or proto-oncogenes are upregulated and converted to oncogenes, potentially leading to cancer

    - Virus may regain virulence once it is in the host, causing diseases
    - Virus may evoke an immune response which makes subsequent repeated therapies not possible as virus would be destroyed by the host immune system
    - Viruses are host specific → Can only target specific cells but not non-host cells ⇒ Cannot use viral vectors for non-host cells
- Advantages of **ex-vivo** approach
    - Target cells easily removed
    - Can screen for stable expression of normal functional allele, tumour formation or presence of infectious viruses before transfer
        - Ensure that there is no insertional mutagenesis before reintroducing cells into patient
    - No introduction of retrovirus into patient
        - May trigger immune response
        - Fear of virus regaining ability to cause disease due to genetic recombination events + Fear of insertional mutagenesis
- Disadvantages
    - Short life span of T cells ⇒ Production of ADA is transient despite integration ⇒ Regular infusion of recombinant T lymphocytes necessary
- Alternatives:
    - Use **haematopoietic stem cells** for longer lasting results
        - Stem cells can undergo extensive proliferation and self-renewal via **mitosis** to produce genetically identical cells ⇒ Ensures constant supply of T cells with ability to produce ADA

Cystic Fibrosis
Description:

- Autosomal recessive
- Deletion mutation of 3 nucleotides in the CFTR gene on **exon 10** of **chromosome 7** ⇒ Loss of phenylalanine in protein ⇒ **Loss of function** mutation ⇒ Defective/Non-production of **cystic fibrosis transmembrane conductance regulator** (CFTR)
- Normal CFTR gene codes for functional CFTR that is an ATP-gated channel protein responsible for transport of Cl^-
- **Defective/missing** CFTR ⇒ **Cl^-** not transported out of epithelial cells into lumen of air cavity ⇒ Na^+ not transported out ⇒ More negative water potential in cell ⇒ Water retained in the cell ⇒ Mucus in lumen becomes thick and undiluted
  ⇒ Blockage/congestion of airway
- Effects of disease:
    - Thick mucus lining the lung lumen ⇒ Severe breathing difficulty + Reduced gaseous exchange
    - Thick mucus in respiratory tract traps bacteria and dirt ⇒ Bacteria growth in respiratory tract ⇒ Chronic lung infection ⇒ Death
    - Pancreatic duct choked by thick mucus preventing release of enzymes ⇒

Indigestion
    - No reabsorption of NaCl by sweat glands ⇒ Very salty and copius sweat production
    - Thick mucus layer in intestines ⇒ Reduced absorption of digested food

Conventional Treatment:

- Daily physiotherapy to physically remove mucus
- Daily medication (e.g. antibiotics, pancreatic enzymes) → Improve quality of life

Gene Therapy:

- **In vivo** approach
- **Non-viral** delivery system → **Liposome transfection**
- Method:
    - Normal functional CFTR allele from chromosome 7 isolated from normal individual inserted into plasmid vector
    - Plasmid vector encapsulated by liposome and packaged in an aerosol dispenser
    - Aerosol dispenser delivers aerosol of liposomes into lungs of patient
    - Liposome fuses with membrane of lung epithelium and delivers normal, functional allele into affected cells
    - Plasmid containing CFTR allele enters nucleus and is transcribed and translated into
    - Functional CFTR proteins which are embedded into plasma membrane
- Advantages of non-viral mediated system
    - Non-pathogenic ⇒ No safety concerns over virus regaining virulence
    - No concerns of triggering unwanted immune reactions in patients ⇒ Repeated treatment is possible
    - No limit to size of allele to be inserted
- Disadvantages of non-viral mediated system
    - Treatment is transient as lung epithelial cells are constantly shed ⇒ Requires regular treatment with aerosol spray
    - Low transfection efficiency as no mechanism for attaching and entering cells
    - Low rate of stable integration as no integrase
    - Low gene expression
- Alternatives: Viral delivery system
    - Use modified adenovirus as a vector with glycoproteins that can bind to epithelial lung cell receptors
        - Will not cause cancer as no integration into host cell genome
        - High transfection efficiency and gene expression
        - Disease causing genes must be removed
            - Genes needed for assembly of virus/viral replication

Factors that keep gene therapy from becoming an effective treatment for genetic diseases (possible essay)

1. **Short lived nature** of gene therapy
    - Due to natural cell death and replacement of cells containing normal functional allele (overcome by stem cells but not always possible)
    - Due to difficulty in stably integrating normal, functional allele into genome ⇒ Normal functional allele gradually lost with each subsequent cell division
    - Requires multiple rounds of gene therapy
2. **Multigene disorders** difficult to treat
    - Caused by combined effects of several genes
    - Gene therapy can currently only treat single gene disorders
3. **Precise level of expression** of normal gene required for some diseases for treatment to be effective (e.g. Thalassemia)
    - Currently difficult to regulate gene expression levels
4. Viral vectors may trigger **immune response**
    - May cause allergic, inflammatory or toxicity responses
    - May destroy viral vector before normal functional allele is delivered to target cells
    - Subsequent treatments may elicit stronger and faster responses by the immune system ⇒ Ineffective
5. Viral vectors are **host specific**
    - Can only target specific cells ⇒ Unable to target non-host cells
6. Viruses may recover its ability to cause diseases in the host due to genetic recombination events
7. Retroviruses may induce **insertional mutagenesis** ⇒ Cancer
    - Random integration of viral genome into host cell genome may
        - Inactivate tumour suppressor gene or
        - Activate proto-oncogene by inserting strong regulatory sequences

Mention before social and ethical arguments FOR/AGAINST use of gene therapy:
Gene Therapy = Introduction of normal, functional allele into cells with defective mutant alleles so that cells can produce a functional protein

- Somatic gene therapy affects only that individual while germline gene therapy affects offspring and successive generations but not the individual

Social and ethical arguments FOR gene therapy

- Potential for treating desperately ill patients especially where conventional treatment methods have failed → May be the only effective way to treat patients
- Scientific community has right to free inquiry → Gene therapy helps to study gene function
- Somatic gene therapy alleviates genetic defect present in individual alone without

impacting genetic information transmitted to next generation
- Germline therapy:
    - Offers a true complete cure, not just palliative/symptomatic
    - May be the only effective way of addressing some genetic diseases where the disease affects an extensive or inaccessible area of the body
    - Avoids risk and expense of somatic cell therapy for future generations
    - Prospective parents that are sufferers/carriers have an option of having normal children without fear of passing on the disease

Social and ethical arguments AGAINST gene therapy

- New and unreliable ⇒ Long term effects unknown
- Many gene therapy candidates are children who are too young to comprehend the consequences of undergoing gene therapy treatment ⇒ Could become unwitting participants
- Very expensive and thus may only be available to those who can afford it ⇒ Income inequality + Unequal resource allocation as research funding could be put to better use
- Germline therapy:
    - Difficult to follow patients in long-term clinical research as they need to be under surveillance for decades to monitor the long-term effects
    - Opens the door to attempts at genetic enhancement not associated with disease ⇒ Exacerbates social and income inequality as well as increased social discrimination, decreased tolerance for human diversity
    - Involves research on early embryos which may be considered by some as living ⇒ Death of embryos during the process of gene therapy may be considered unethical as it is considered taking a life
    - Creates generations of unconsenting research subjects

	Advantages	Disadvantages
Retrovirus	• High transfection efficiency • Stable gene expression as able to integrate normal functional allele into host cell genome using integrase	• Random integration ⇒ May cause **insertional mutagenesis** • May trigger immune response (overcome by lentivirus) • Limit to size of insert • Virus is dangerous and may regain ability to cause diseases due to genetic recombination events

		• Only infects dividing cells (overcome by lentivirus)
Adenovirus	• High transfection efficiency • Mechanism to inject DNA through nuclear pore into nucleus • No integration ⇒ No insertional mutagenesis • Infects both dividing and non-dividing cells	• No integration into genome ⇒ Transient gene expression ⇒ DNA not passed on to daughter cells • Triggers immune response • May regain ability to cause diseases due to genetic recombination events • Limit to size of insert
Adeno-associated virus	• High transfection efficiency • Non-pathogenic • No immune response • Inserts into specific site in chromosome 19 ⇒ No random integration + No insertional mutagenesis • Infects dividing and non-dividing cells	• Limit to size of insert
Liposomes	• Non-pathogenic • No immune response • No limit to size of insert • Can be targeted for specific cells through addition of specific glycoproteins to liposome membrane	• Do not target specific cell type ⇒ Low transfection efficiency • Low rate of stable integration ⇒ Short-lived gene expression of normal functional allele