Gene therapy
What is gene therapy?

More than 3000 specific conditions are known to be inherited and caused by mutations in the genes spread over the human genome.

**Gene therapy:**

- A potential approach to the management of genetic disorder
- Technique for “correcting” defective genes responsible for disease development

[Diagram showing gene insertion and inactivation]
Prerequisites for successful gene therapy

- A candidate gene to be replaced, or a strategy for repairing or silencing a faulty gene (genetic background of disease has to be identified)

- A delivery mechanism for getting the new gene:
  - into the **correct tissue**
  - in the **correct amounts**
  - for **sufficient period of time**

- Beneficial expression of the new gene

- Benefits of the new gene that must outweigh any risks of the gene and/or the gene delivery system
Approaches for gene delivery

In vivo gene therapy

1. copies of therapeutic gene are inserted into viral DNA, liposome, or in form of plasmid DNA
2. genetically-altered DNA is inserted into patient's body by cell-specific direct tissue injection
3. Inside the body, the inserted DNA is incorporated into the cells of the specific tissue it was injected into. These cells now encode and produce the needed protein encoded by the inserted gene

Ex vivo gene therapy

1. copies of therapeutic gene
2. gene inserted into viral DNA
3. cultured cells are infected with genetically-altered virus
4. patient's sample target cells are now genetically altered with therapeutic gene
5. Inside the body, the genetically altered cells produce the desired proteins encoded by the therapeutic DNA

Approaches to correct a faulty gene

- replacement
- repair
- gene silencing

Homologous Recombination:
- DNA template provided for homologous recombination
- Gene Insertion or Gene Repair

Non Homologous End Joining (NHEJ):
- Loss of a few base pairs
- Gene Inactivation
Replacement of faulty gene

For diseases caused by „loss-of-function” mutations or gene deletions

It may be sufficient to simply „add-in” a functional version of the gene (gene for appropriate clotting factor in haemophilia, for chloride transporter in cystic fibrosis)

VECTOR

stem cells provide new generations with the appropriate gene

Duchenne muscular dystrophy is an X-linked pathology due to the absence of dystrophin in muscle fibers.

It affects mainly children (2-6 years), more prominent in boys.

Symptoms: addling walk, arched back, enlarged calves, weakening muscles in legs and arms, abnormal joints, breathing and heart problems.

http://mda.org/disease/duchenne-muscular-dystrophy/causes-inheritance
Repair of faulty gene example – muscular dystrophy

Strategy: **antisense oligonucleotides**

„exon skipping” strategy for the treatment of Duchenne muscular dystrophy

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Patient with a deletion of exon 50

(a) The absence of exon 50 in the dystrophin gene leads to an out-of-frame mRNA creating a premature stop codon in exon 51, thus aborting dystrophin synthesis during translation.

(b) Using an **antisense oligonucleotides** (AO) targeting exon 51, this exon is skipped during splicing. This restores the open reading frame of the transcript and allows the synthesis of an internally deleted dystrophin.
Repair of faulty gene

Strategy: **homologous recombination**
- natural repair mechanism that can occur during cell division
- repair of double-stranded chromosomal DNA in which both strands have broken

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**potential treatment of X-SCID:**
- introduction of an engineered DNAse into the bone marrow cells along with a healthy copy of the IL2R gene
- Enzyme specifically cleaves the DNA of the mutant IL2R gene and the healthy gene acts as a template for its repair
Faulty gene silencing

non-coding sequences

antisense oligonucleotides (DNA)  siRNA (RNA)
Antisense oligonucleotides

Short fragments of single strand, chemically modified DNA nucleotides (oligonucleotides) (13-25 nucleotides long), complementary to a given mRNA.

act by:

a) inhibition of protein translation by disruption of protein assembly

b) modulation of splicing

c) utilise RNase H enzymes to degrade mRNA

Rnase H – non-specific endonuclease, catalyzes the cleavage of RNA via an hydrolytic mechanism
Oblimersen -
Bcl2 antisense - melanoma

The US Food and Drug Administration (FDA) gave oblimersen orphan drug status for malignant melanoma in August 2000.

It comprises a phosphorothioate backbone linking 18 modified DNA bases. **Oblimersen targets the first six codons of Bcl-2 mRNA.**

By reducing the amount of anti-apoptotic Bcl-2 protein in cancer cells, oblimersen may enhance the effectiveness of conventional anticancer treatments.
Specific inhibition of gene expression by double-stranded RNA, which stimulates the degradation of a target mRNA

**Nobel prize 2007**

Andrew Fire  Craig Mello

**Delivery of siRNA to mammalian cells**

Chemically synthesised siRNA – short inhibition

siRNA-encoding vectors – long-term inhibition

**Mechanisms of RNA interference**

**Dicer** – an enzyme that cleaves long dsRNA into short double stranded fragments of ~20 nucleotide siRNAs

passenger strand is degraded and the guide strand is incorporated into the RNA-induced silencing complex (RISC)

guide strand pairs with a complementary sequence in a messenger RNA molecule and induces cleavage

Recent progress in clinical trials of siRNA therapy is promising, with the majority of those studies to date focusing on the treatment of cancers and ocular conditions.

<table>
<thead>
<tr>
<th>Company</th>
<th>Drug</th>
<th>Delivery route</th>
<th>Target</th>
<th>Vehicle</th>
<th>Disease</th>
<th>Phase</th>
<th>Status</th>
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<td>Santaris</td>
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<td>SC</td>
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<td>Opko Health</td>
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<td>IVT</td>
<td>VEGF</td>
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<td>PF-655</td>
<td>IVT</td>
<td>RTP801</td>
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<td>Nebulization or intranasal</td>
<td>RSV Nucleocapsid</td>
<td>Naked siRNA</td>
<td>RSV</td>
<td>IIIb</td>
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<td>CEQ508</td>
<td>Oral</td>
<td>Beta catenin</td>
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<td>KRASG12D</td>
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<td>PLK1</td>
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<td>Alnylam/Tekmira</td>
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<td>KSP and VEGF</td>
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<td>ALN-TTR01</td>
<td>IV</td>
<td>TTR</td>
<td>SNALP</td>
<td>TTR-mediated amyloidosis (ATTR)</td>
<td>I</td>
<td>Ongoing</td>
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<td>University Duisburg</td>
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<td>Bcr-Abl</td>
<td>Anionic liposome</td>
<td>CML</td>
<td>I</td>
<td>Completed</td>
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<td>Silence Therapeutics</td>
<td>Am027</td>
<td>IV</td>
<td>PKN3</td>
<td>siRNA-lipoplex</td>
<td>Advanced solid cancer</td>
<td>I</td>
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<td>Quark Pharma</td>
<td>15NP</td>
<td>IV</td>
<td>P53</td>
<td>Naked siRNA</td>
<td>AKI and DGF</td>
<td>II</td>
<td>Ongoing</td>
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<td>Calando Pharma</td>
<td>CALAA-01</td>
<td>IV</td>
<td>RRM2</td>
<td>Cyclodextrin nanoparticle, TF, and PEG</td>
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<td>I</td>
<td>Ongoing</td>
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<td>FANG vaccine</td>
<td>IV</td>
<td>Ex vivo IV</td>
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<td>iPSiRNA</td>
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<td>Transfection</td>
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<td>IV</td>
<td>Ex vivo transplant</td>
<td>HIV Tat and Rev</td>
<td>Lentivirus</td>
<td>I</td>
<td>Ongoing</td>
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</table>

Burnett & Rossi, Chem Biol, 2012
Despite the huge therapeutic potential, siRNA based therapeutics is yet to be approved by EMA or FDA. To move siRNA therapeutics into the clinic, two major bottlenecks must be overcome: abrogation of off-target silencing effects and efficient delivery of siRNA.

The specificity of RNAi is not as robust as it was initially thought to be. Introduction of siRNA can result in off-target effect, i.e. the suppression of genes other than the desired gene target, leading to dangerous mutations of gene expression and unexpected consequences. Overall, therapeutic siRNA must be carefully designed. A combination of computer algorithms and empirical testing is also encouraged to allow effective design of potent siRNA sequences and minimize off-target effect.

SiRNA are susceptible to nuclease degradation and cannot be systemically administered. Chemical modification of siRNA can improve its resistance against nucleases. In addition, siRNA is negatively charged, hydrophilic macromolecule with poor membrane permeability, a delivery agent is therefore required to facilitate the cellular uptake of siRNA and to protect the siRNA from premature degradation. Although new materials and delivery systems are being investigated to enhance the delivery efficiency, approval procedures could be hindered by the complicated formulation.

On the other hand, eyes and lungs are promising tissues for local delivery of naked siRNA, especially the former, which is reflected by the high number of clinical trial studies targeting this site.
Prerequisites for successful gene therapy

- A candidate gene to be replaced, or a strategy for repairing or silencing a faulty gene (genetic background of disease has to be identified)

- A delivery mechanism for getting the new gene:
  - into the correct tissue
  - in the correct amounts
  - for sufficient period of time

- Beneficial expression of the new gene

- Benefits of the new gene that must outweigh any risks of the gene and/or the gene delivery system
Gene delivery systems - VECTORS

**non-viral**

- „naked” DNA
- lipoplexes
- viroplexes (lipoplexes enhanced in proteins from viral capsids)
- Chimeric proteins
- Complexes with chemical vehicles

**viral**

- RNA
  - Retroviral (including Lentiviral)
- DNA
  - Adenoviral
  - AAV
  - Herpes

No risk of infection by the vector
Much larger DNA sequences can be incorporated
Plasmids - the main tools of gene therapy

Organisation of a typical plasmid vector

DNA cloning

Region into which DNA can be inserted

Plasmid cloning vector

DNA is cut with EcoRI at arrows.

Resulting DNAs have sticky (complementary) ends.

DNA is spliced by complementary base pairing and sealed with DNA ligase

DNA to be inserted
Transformation of bacteria

Figure 8–31. Molecular Biology of the Cell, 4th Edition.
Expression of foreign genes in eucaryotic cells requires the eucaryotic promoter in a plasmid vector.

- **Viral promoters**: CMV, SV40

- **Eucaryotic promoters:**
  - constitutive
    - non-selective: b-globin
    - tissue-specific
  - inducible

- Complex
‘Naked’ DNA

- low efficiency related to:
  - negative charge of both DNA and cell membrane (electrostatic barrier)
  - rapid break down of DNA by DNAses in the blood and tissues

- requires large amounts of DNA

- low immunogenicity

- may be of use for the delivery of ‘genetic vaccines’, which would only require the expression of relatively low amounts of protein
DNA vaccines

- the first DNA vaccine was licensed in 2005, for the protection of horses from West Nile virus
- compared with protein, DNA is much simpler and easier to produce and purify, more stable in storage
- Low level of antigen expression (immune response may be insufficient to provide protection)
- Some of the DNA get taken up into cells, which then express the antigen on the cell surface and stimulate immune response

Efforts to:

- Immunostimulatory adjuvant
- improve DNA delivery technique to enhance antigen expression

Safety and ethical concerns:

- risk of formation anti-DNA antibodies
- possible incorporation into the host’s genome
- possible adverse effects of the long term expression of a foreign antigen
Lipoplexes

Lipoplex – liposome/DNA complex
- Artificial lipids spheres with an aqueous core formed by sonicating a mixture of water, lipid and DNA
- condensation of long DNA molecule into more compact form
- Liposome protects DNA from breakdown by DNAses
- if made with cationic lipids its positive charge overcomes electrostatic barrier of the cell membrane

- Viroplexes (lipoplexes enhanced in proteins from viral capsids)
Chimeric proteins

DNA enter target cells by chemically linking to a genetically engineered bifunctional protein

Essentially two proteins linked together:

- a DNA-binding protein, such as protamine, to bind and condense the DNA
- a protein, such as ferritin, that will bind to specific receptors on the target cell membrane and facilitate entry into the cell

Can be designed to target particular cell types
# Non-viral vectors - summary

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<th>Vector</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td>Non-viral vectors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naked DNA</td>
<td>No size limitation, very simple to produce</td>
<td>Poor stability <em>in vivo</em>, very inefficient transfection, risk of anti-DNA antibodies</td>
</tr>
<tr>
<td>Liposomes</td>
<td>No size limitation, simple to produce, improved stability and transfection efficiency, properties can be modified to improve cell selectivity, for example</td>
<td>Low transfection efficiency</td>
</tr>
<tr>
<td>Chimeric proteins</td>
<td>Can be designed to target particular cell types</td>
<td>Very ineffective in studies to date</td>
</tr>
<tr>
<td>Artificial chromosome</td>
<td>No size limitation, well tolerated within cells, stable gene expression</td>
<td>Difficult to make, very difficult to get into cells</td>
</tr>
</tbody>
</table>
Viral vectors

- In many ways ideal gene therapy vectors
- evolved efficient mechanism for inserting their ‘foreign’ genetic material into the host cell and for persuading the host cell to express that genetic material
- stringent size restrictions of DNA to be inserted

Precautions:

- the virus must be rendered replication-deficient, to prevent its uncontrolled proliferation, which would result in infection of the human host
- the virus should not provoke an immune response from the host, as this itself can cause tissue damage
Native entry mechanisms of unmodified viral vectors

- Adenovirus binds to its receptor **CAR** (coxsackie and adenovirus receptor) through its fibre knob.

- Integrins interact with the capsid protein at the base of the fibre and facilitate cell entry by endocytosis.

- AAV2 first binds to **heparan-sulphate proteoglycan (HSPG)** and then to the co-receptor, which can be either an integrin, hFGFR or HGFR. The virus is internalized by endocytosis.

- Other AAV serotypes either resemble AAV2 in its heparin binding or use different primary receptors e.g. **sialic acid**.

- The host range of retroviral vectors is determined by the interaction of the viral envelope protein (Env) and the cellular receptor. 

  - **Fusion between the lipid membranes of the virus and the host cell**, following which the viral nucleocapsid is released into the cytoplasm.

Waehler et al., Nature Reviews Genetics, 2007
Retroviral vectors

infectious cycle of retrovirus

- infect mostly dividing cells
- long-term expression due to integration into cellular genome
- risk of insertional mutagenesis
- Especially useful for stem cells gene therapy, like in SCID trials
Lentiviral vectors – a special type of retroviral vectors

- developed from highly pathogenic HIV virus
- for non-dividing cells
- integrate into genome
  - The vector, now called a provirus, remains in the genome and is passed on to the progeny of the cell when it divides.
- widespread clinical application after rigorous safety testing
Adenoviral vectors

- Adenoviruses - DNA parvoviruses associated with the common cold in human
- Infect both dividing and non-dividing cells

- Genome consists of **double-stranded linear DNA** with ITR sequences at each end
  - Early genes – responsible for viral gene transcription, DNA replication, host immune suppression and host cell apoptosis
  - Late genes – coding proteins required for virus assembly

- E1 early gene is essential for the subsequent adenoviral gene expression

- Vectors provide high but transient transgene expression

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Adeno-associated vectors (AAVs)

- Linear single-stranded DNA parvoviruses non-pathogenic to humans
- Replication-deficient and have traditionally required co-infection with a helper adenovirus or herpes virus for replication and productive infection
- Can replicate with and without incorporation into the host genome
- Infect both dividing and non-dividing cells
- The best safety profile from all viral vectors

ITR – necessary in cis:
- Initiation of replication
- Packaging signal
- Integration into genome
## Viral vectors - summary

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<th>Vector</th>
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<th>Disadvantages</th>
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<tbody>
<tr>
<td><strong>Viral vectors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retroviruses, e.g. murine</td>
<td>Non-pathogenic in humans, much prior experience, efficient transfection,</td>
<td>Risk of insertional mutagenesis, only targets dividing cells, small insert size</td>
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<tr>
<td>leukaemia virus</td>
<td>stable gene expression</td>
<td></td>
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<tr>
<td>Lentiviruses, e.g. HIV</td>
<td>Stable gene expression, will target non-dividing cells</td>
<td>Risk of insertional mutagenesis, risk of virulent reversion</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Much prior experience, easy to grow, targets both dividing and non-dividing</td>
<td>Transient gene expression, immunogenic, risk of virulent reversion</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Non-pathogenic in humans, stable gene expression, will target non-dividing</td>
<td>Risk of insertional mutagenesis, immunogenic (but less than adenovirus), small</td>
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<tr>
<td>Herpes simplex virus (HSV)</td>
<td>Readily enters CNS, inserts up to 30 kb</td>
<td>insert size (&lt; 4.5 kb)</td>
</tr>
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</table>

**Integrating**
- retroviral
- lentiviral
- AAV

**Non-integrating**
- adenoviral
- HSV

**Integration depends on:**
- LTR sequences and integrase (retroviruses)
- ITR sequences and rep proteins (AAV)
Targeting genes to particular cells

Accurate temporal and spatial regulation of gene expression is crucial for proper control of protein level and cell fate

Two ways of targeting gene therapy:

- **anatomically** via the route of administration
- **molecularly** using the molecular properties of the target cells and/or the vector
  - transcriptional regulation
  - post-transcriptional regulation
Routes of administration

- intratumoral (cancers, when the location is known and accessible)
- subcutaneous
- intravenous
- intramuscular
- intradermal
- intraperitoneal
- aerosol inhaled into the lungs (e.g. cystic fibrosis)
- by bone marrow transplantation (e.g. for SCID)

Depends on:
- nature of the disease
- the type of gene
- amount of DNA to be delivered
- type of vector

- e.g. a gene that causes apoptosis, in a nonselective vector, has to be administered straight into a tumour,

however, if the same gene could be delivered in a vector which specifically targets the tumour cells, it could be delivered intravenously.
Molecular targeting – Transcriptional regulation

Introduction of the therapeutic gene to the given cells should provide the level of expression which will restore the production of therapeutic protein to normal values or will provide therapeutic efficacy despite not fully physiological expression.

In numerous diseases the expression of therapeutic genes has to be kept at certain level for some time.

- Cell-specific promoters
- Regulatable promoters: Artificial systems
  - Tetracycline-regulated system
  - Other ligand-induced systems
- Physiologically regulatable vectors
  - Hypoxia-regulated vectors
  - Oxidative stress-regulated vectors
- Combined promoters – cell specific regulation
The cell-specific promoters are used to restrict the expression to selected cell types in order to maximize the therapeutic effect or to reduce the side effects of the “off-line” expression.

- **endothelial-specific** - Flk-1 (receptor for VEGF), Tie-2 (receptor for Angiopoietin)
- **keratinocyte specific** - Keratin 14
- **tumor-specific** - TERT, Cox-2 and BIRC5 - enhance the expression of dependent genes in many tumor types but not (or very modestly) in normal tissues

**Limitation**: many genes, claimed initially to be expressed in one cell type, are in fact more ubiquitously active
**Tet-ON system** - When doxycycline/Tet is present in the microenvironment, the tTS dissociates from TetO, relieving the transcriptional suppression. At sufficient concentrations, doxycycline also interacts with reverse tTA (rtTA), and by changing its conformation allows it to bind to TetO and activate the transgene expression.

**Tetracycline (Tet)**

Tet-controlled transcriptional silencer (tTS) - fusion protein of Tet repressor (tetR) and the KRAB-AB domain of the Kid-1 protein

rtTA, reverse tetracycline-controlled transactivator

operator sequences (TetO)
Mechanisms of blood vessels formation – involvement of heme oxygenase - 1

vasculogenesis
Formation of new blood vessels *de novo* from vascular progenitor cells

angiogenesis
Formation of new blood vessels from pre-existing ones

![Diagram showing the mechanisms of vasculogenesis and angiogenesis with involvement of heme oxygenase (HO-1)](image)
Murine model of angiogenesis – hind limb ischemia

After: Hiroshi Niiyama, Ngan Huang, Mark Rollins, John Cooke, Stanford University
Application of regulatable vectors - hypoxia-regulated pro-angiogenic therapy

the plasmid vector harboring human HO-1 cDNA which expression is regulated by 3 HRE-sequences

HRE- hypoxia responsive element

HIF1α

stabilization

proteasomal degradation

HO-1

hypoxia

O₂, α-ketoglutarate, Fe²⁺-dependent prolyl hydroxylases

plasma membrane

nucleus

Expression

HIF1α

3 x HRE minCMV HO-1 cDNA

HRE- hypoxia responsive element

Jazwa et al., Gene, 2013
Application of regulatable vectors
- hypoxia-regulated pro-angiogenic therapy

About 10–15 min before vessel ligation, pHRE-empty or pHRE-HO-1 plasmid was delivered into the left gastrocnemius muscle

Post-ischemic blood flow is improved in mouse hindlimbs overexpressing HO-1

- \( \downarrow \) \( \text{H}_2\text{O}_2 \)-induced cell death of endothelial cells
- \( \uparrow \) proliferation of endothelial cells
- \( \downarrow \) post-ischemic inflammation
- \( \downarrow \) post-ischemic muscle cell death
- \( \uparrow \) post-ischemic blood flow
- \( \uparrow \) regenerative potential of muscles

Jazwa et al., Cardiovasc Res, 2013
Molecular targeting – post-transcriptional regulation (microRNA)

MicroRNAs - small, ~20 nucleotides long RNA which target specific sequences in mRNA often expressed in a cell-specific manner.

MicroRNA targeted vectors may allow cell-specific regulation of gene expression - negative expression control over the introduced transgene.
Molecular targeting - post-transcriptional regulation (microRNA)

- repression of the transgene in the presence of the miRNA
- a bidirectional lentiviral vector including miRNA-142-3p binding site in the 3’-UTR of the GFP reporter cassette
- mir-142-5p and mir-142-3p are enriched in hematopoietic cells

Transgene (GFP) expression from vectors incorporating target sequences for miRNA-142-3p was effectively suppressed in hematopoietic lineages, whereas expression was maintained in nonhematopoietic cells.

- a way to prevent the immune system from rejecting the transgene

Hematopoietic lineage cells were marked by CD45 immunostaining (red) in all organs
Summary - regulation of gene expression in gene therapy

Levels of gene expression regulation in gene therapy

- Transcriptional regulation
  - Cell/tissue-specific vectors
    - Tumor-specific
    - Cell/tissue-specific
  - Condition-regulatable vectors

- Post-transcriptional regulation
  - MicroRNA-regulated vectors (harbouring 3'UTR)

- Gene targeting (recombination dependent)
  - Cre/loxP system*
    - Deletion or inversion of target gene
    - Gene replacement (targeted mutagenesis)
  - *Cell/tissue-specific and/or condition-regulatable vectors for expression of Cre may be used

- Artificial systems (ligand regulatable)
  - Tetracycline-regulated (Tet-On/Off systems)
  - Prostaglandin-regulated
  - Rapamycin-regulated

- Physiologically regulatable vectors
  - Hypoxia-regulated vectors (harbouring HRE)
  - Electrophile-regulated vectors (harbouring ARE)

- Combined - cell/tissue-specific regulatable vectors
  - Cell/tissue-specific regulated by ligand
    - *E.g. pancreas-specific enhancer induced by tetracycline
  - Cell/tissue-specific physiologically regulated
    - *E.g. heart promoter activated only in hypoxia

*DNA sequence-specific binding

Jazwa et al. Gene 2013
Up to date, cancer is by far the most common disease treated by gene therapy.
Adenoviral vectors have been the most commonly used gene transfer vectors in clinical trials.
### Some Gene Therapy Successes

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Disease type</th>
<th>Patients benefiting</th>
<th>First publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-SCID</td>
<td>Immunodeficiency</td>
<td>17/20</td>
<td>2000</td>
</tr>
<tr>
<td>ADA-SCID</td>
<td>Immunodeficiency</td>
<td>26/37</td>
<td>2002</td>
</tr>
<tr>
<td>Adrenoleukodystrophy</td>
<td>Neurologic</td>
<td>2/4*</td>
<td>2009</td>
</tr>
<tr>
<td>Leber’s congenital amaurosis</td>
<td>Blindness</td>
<td>28/30</td>
<td>2008</td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>Immunodeficiency</td>
<td>8/10</td>
<td>2010</td>
</tr>
<tr>
<td>β-thalassemia</td>
<td>Hemoglobinopathy</td>
<td>1/1</td>
<td>2010</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>Coagulation</td>
<td>6/6</td>
<td>2011?</td>
</tr>
</tbody>
</table>

*Includes a patient treated too recently to see benefit*
Gene therapy for primary immunodeficiencies

- Primary immunodeficiencies are **due to blood cell defects** and can be treated with:
  - transplantation of normal hematopoietic stem cells (**HSC** from another person (allogeneic))
  - genetically corrected **patient's autologous HSC** (avoid the immunologic risks)

- Recent clinical trials using gene therapy have led to immune restoration in patients with:
  - X-linked severe combined immune deficiency (**XSCID**),
  - adenosine deaminase (**ADA**) - deficient SCID
  - chronic granulomatous disease (**CGD**)  

- However, severe complications arose in several of the patients in whom the integrated retroviral vectors led to **leukoproliferative disorders**.

- New approaches using safer integrating vectors or direct correction of the defective gene underlying the PID are being developed.
Successful treatment of ADA-SCID

Gene therapy using peripheral blood lymphocytes (PBLs) or HSCs

Unlike the experience with X-SCID, **retroviral transduction of HSC continues to show high therapeutic efficacy in ADA-SCID without the development of leukemia**

- PBL gene therapy was able to restore T-cell functions after discontinuation of ADA enzyme replacement therapy, but only partially corrected the purine metabolic defect.
- The development of improved HSC gene transfer protocols, combined with low intensity conditioning, allowed full correction of the immunological and metabolic ADA defects, with clinic benefit.

Aiuti et al., Immunol Ires, 2009
**AAV vectors for the treatment of inherited blindness**

**Leber’s congenital amaurosis (LCA)** - rare form of inherited blindness caused by progressive loss of rod and cone function due to the lack of the RPE65 isomerase, which is required to form light sensitive pigment.

In 2008, three groups reported success in LCA patients using the AAV2 vector.

- Local (subretinal, an immune privileged site) administration of a vector expressing RPE65 led to gain of light sensitivity and, in some cases, of vision, in patients with LCA.

Treatment involved the inferior retina in patient 1, superior retina in patient 2, and the far temporal retina in patient 3.

(Bainbridge et al., 2008; Cideciyan et al., 2008; Maguire et al., 2008)
Gene therapy strategies for HIV/AIDS

The human immunodeficiency virus (HIV)

- a lentivirus that causes the acquired immunodeficiency syndrome (AIDS), a condition of progressive failure of the immune system

- HIV infects:
  - helper T cells (specifically CD4+ T cells)
  - macrophages
  - dendritic cells

In the absence of an effective vaccine and lack of a complete cure, gene therapy approaches to control HIV infection offer feasible alternatives.

HIV susceptible cells that continuously arise from the bone marrow source.
Gene therapy strategies for HIV/AIDS

gene therapy approaches employ various anti-HIV therapeutic molecules

Table 2. HIV Gene Therapy Clinical Trials.

<table>
<thead>
<tr>
<th>Gene therapy construct (viral or cellular target)</th>
<th>Gene modified cells</th>
<th>Delivery method</th>
<th>Phase, status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antisense (env mRNA)</td>
<td>Autologous CD4+ T cells</td>
<td>Lentiviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>ZFN (CCR5 gene)</td>
<td>Autologous CD4+ T cells</td>
<td>Adenoviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>shRNA (CCR5 mRNA)</td>
<td>Autologous</td>
<td>Lentiviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>Fusion inhibitor C46 (env protein)</td>
<td>CD34+ HSCs and CD4+ T cells</td>
<td>Retroviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>Fusion inhibitor C46 (env protein)</td>
<td>Autologous of Allogeneic CD34+ HSCs</td>
<td>Retroviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>Endoribonuclease (ACA sequences)</td>
<td>Autologous CD8+ T cells</td>
<td>Lentiviral vector</td>
<td>I, Ongoing</td>
</tr>
<tr>
<td>Transgenic TCR (gag epitope)</td>
<td>Autologous CD4+ T cells</td>
<td>Retroviral vector</td>
<td>I, Ongoing</td>
</tr>
<tr>
<td>Chimeric antigen receptor (gp120 protein)</td>
<td>Autologous CD4+ and CD8+ T cells</td>
<td>Retroviral vector</td>
<td>I-II, Completed</td>
</tr>
<tr>
<td>Antisense (TAR, tat/rev mRNA)</td>
<td>Autologous CD34+ HSCs</td>
<td>Retroviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>Ribozyme (tat/vpr mRNA)</td>
<td>Autologous CD34+ HSCs</td>
<td>Retroviral vector</td>
<td>II, Ongoing</td>
</tr>
</tbody>
</table>
Types of cancer gene therapy

1. Direct attack on tumor cells
   a) transfer of tumor suppressor gene – p53
   b) inhibition of oncogenes
      - antisense therapy
      - ribozymes
      - microRNAs
   c) suicide genes
   d) oncolytic viruses (replication-competent viruses)
2. Harnessing immune response to tumor antigens – tumor immunotherapy
3. Chemoprotection
4. Anti-angiogenic therapy
Harnessing immune response to tumor antigens

- Active immunotherapy – stimulation of immune response to tumor-associated antigens via immunomodulating agents or tumor vaccines
- Passive immunotherapies enhance existing anti-tumor responses and include the use of monoclonal antibodies, lymphocytes and cytokines

History of cancer immunotherapy lasts for more than 120 years. In 1891 William B. Coley injected bacteria into inoperable cancer (bone sarcoma) and observed tumor shrinkage. He is recognized as the """"Father of Immunotherapy"""". Cancer immunotherapy is based on the ability of the immune system to recognize cancer cells and to affect their growth and expansion. Beside the fact that, tumor cells are genetically distinct from their normal counterparts, and should be recognized and eliminated by immune system, the tumor associated antigens (TAAs) are often poorly immunogenic due to immunoediting. This process allows tumor to evolve during continuous interactions with the host immune system, and eventually escape from immune surveillance. Furthermore, tumor microenvironment consists of immunosuppressive cells that release immunosuppressive factors including IL-6, IL-10, IDO, TGFβ or VEGF. Interactions between cancer and stroma cells create network of immunosuppressive pathways, while activation of immune defense is inhibited. A key to successful immunotherapy is to overcome the local immunosuppression within tumor microenvironment and activate mechanisms that lead to tumor eradication. There are two clinical approaches of immunotherapy: active and passive. Active immunotherapy involves stimulation of immune response to tumor associated antigens (TAAs), either nonspecifically via immunomodulating agents or specifically employing cancer vaccines. This review presents the progress and breakthroughs in design, development and clinical application of selected cell-based tumor vaccines achieved due to the generation and development of gene transfer technologies.
How Does Immunotherapy Work?

Tumor cells bind to T-cells to deactivate them

Immunotherapy drugs can block tumor cells from deactivating T-cells
**Immune Boost**

Several methods are showing promise in helping immune sentinels called T cells to attack cancer.

**Checkpoint Inhibitor Drugs**

‘Checkpoint’ proteins block T-cell activity. Inhibitor drugs can release the brakes on T cells at different stages.

The CTLA-4 checkpoint protein prevents dendritic cells from priming T cells to recognize tumours. Inhibitor drugs block the checkpoint.

The PD-1 checkpoint protein prevents T cells from attacking cancer cells. The inhibitor drug allows T cells to act.
gene therapy drugs approval on the market
The history of gene therapy drugs approval on the market

2003 – **Gendicine** (China)
Wild-type p53 gene (adenovirus with correct p53) for the treatment of head and neck cancer.

*There are doubts on the real effectiveness of Gendicine*

2005 – **Oncorine** (China)
E1B-defective adenovirus for the treatment of head and neck cancer.

2011 – **Neovasculogen** (Russia)
VEGF for treatment of peripheral arterial disease (PAD) and its complication critical limb ischemia (CLI)
Gene therapy drugs registered in Europe
Glybera – first registered AAV vector for human gene therapy

Lipoprotein lipase deficiency – disruption of breakdown of fats


AAV1 vector with cDNA of lipoprotein lipase (LPL)

A centralised EU marketing authorisation has been obtained under the name *Glybera*

-around US$1.1 million for Glybera gene therapy
- Gene therapy programs are generating much excitement, but there is little agreement about pricing and how payers will foot the bill
T-VEC – an oncolytic herpesvirus

**Oncolytic Immunotherapy**, using a genetically modified strain of the herpes virus to invade tumors and replicate itself, killing cancer cells along the way and spurring an immune response to double its effect.

Recently registered by EMA and FDA

Talimogene laherparepvec (T-VEC, Amgen)

Large clinical trial – melanoma – virus both shrank the tumors in people with advanced melanoma and extended patient survival by a median of 4.4 months – R.H. Andtbacka et al., J Clin Oncol 33: 2780-2788, 2015

- T-VEC contains deletion of the neurovirulence genes: improves cancer cell selectivity and prevents infections of neurons
Strimvelis intended for the treatment of ADA-SCID

On 1 April 2016 the European Medicines Agency (EMA) has recommended granting a marketing authorisation for the medicinal product Strimvelis in the European Union (EU).

**Target:** patients with adenosine-deaminase-deficient severe combined immunodeficiency (ADA-SCID), who have no matching donor for a stem cell transplant.

**Active substance of Strimvelis:** autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with a retroviral vector that encodes for the human ADA cDNA sequence.

**Strimvelis will be available as dispersion for infusion** (1-10 million cells/ml). After infusion, CD34+ cells engraft in the bone marrow where they repopulate the haematopoietic system with cells, a proportion of which expresses the pharmacologically active levels of the ADA enzyme. Following engraftment in the patient, the effects of the product are expected to be life-long.

In a pivotal clinical study:
- survival rate was 100%, with an average follow-up period of 7 years
- There was also evidence of immune reconstitution with an increase in CD3+ T cells as well as T cell subsets.
- The most common side effects are pyrexia, increased hepatic enzyme levels, autoimmune reactions, such as anaemia, neutropenia, and autoimmune haemolytic anaemia, aplastic anaemia and thrombocytopenia.

gene therapy is a technique for “correcting” defective genes responsible for disease development

Approaches for gene delivery: ex vivo, in vivo gene therapy
Approaches to correct a faulty gene: replacement, repair, silencing

Accurate temporal and spatial regulation of gene expression is crucial for proper control of protein level and cell fate

Two ways of targeting gene therapy:
- anatomically via the route of administration
- molecularly using the molecular properties of the target cells and/or the vector

successful trials on the treatment of ocular diseases and inherited immune deficiencies are particularly encouraging and have raised hopes that human gene therapy as a standard treatment option will finally become a reality