Gene therapy
What is gene therapy?

About half a dozen of defective genes in hidden state (heterozygous)

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
Prerequisites for successful gene therapy

- A candidate gene to be replaced, or a strategy for repairing or silencing a faulty gene
- 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
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. target cells removed from patient
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)

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

(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

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

http://mda.org/disease/duchenne-muscular-dystrophy/causes-inheritance
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

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

The 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.
siRNA

Specific inhibition of gene expression by double-stranded RNA, which stimulates the degradation of a target mRNA.

Nobel prize 2007
Andrew Fire  Craig Mello

Mechanisms of RNA interference

- Double-stranded RNA (dsRNA) is cleaved by Dicer into small interfering RNA (siRNA).
- siRNA is incorporated into the RNA-induced silencing complex (RISC).
- RISC targets and degrades mRNAs complementary to the guide strand of siRNA.

Delivery of siRNA to mammalian cells

Chemically synthesised siRNA - short inhibition
siRNA-encoding vectors - long-term inhibition

## siRNA therapeutics in clinical trials

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<tr>
<th>Company</th>
<th>Drug Description</th>
<th>Delivery Route</th>
<th>Target Description</th>
<th>Vehicle Description</th>
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<td>Santaris</td>
<td>SPC3649 (LNA)</td>
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<td>III</td>
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<td>Quark/Pfizer</td>
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<td>Ophthalmic drops</td>
<td>ADRB2</td>
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<td>Intraocular Pressure</td>
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<td>ExcellairTM</td>
<td>Inhalation</td>
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<td>Alnylam/Cubist</td>
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<td>Nebulization or intranasal</td>
<td>RSV Nucleocapsid</td>
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<td>Marina Biotech</td>
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<td>Apo B</td>
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<td>Alnylam</td>
<td>ALN-TTR01</td>
<td>IV</td>
<td>TTR</td>
<td>SNALP</td>
<td>TTR-mediated amyloidosis (ATTR)</td>
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<td>University Duisburg</td>
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<td>Silence Therapeutics</td>
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<td>IV</td>
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<td>15NP</td>
<td>IV</td>
<td>P53</td>
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<td>IV</td>
<td>RRM2</td>
<td>Cyclodextrin nanoparticle, TF, and PEG</td>
<td>Solid tumors</td>
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<td>Ongoing</td>
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<td>Gradalis Inc.</td>
<td>FANG vaccine</td>
<td>IV</td>
<td>Ex vivo IV</td>
<td>Furin and GM-CSF</td>
<td>Electroporation</td>
<td>II</td>
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<td>Duke University</td>
<td>iPSiRNA</td>
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<td>Ex vivo intradermal injection</td>
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<td>City of Hope/Benitec</td>
<td>Tat/Rev shRNA</td>
<td>IV</td>
<td>Ex vivo transplant</td>
<td>HIV Tat and Rev</td>
<td>Lentivirus</td>
<td>I</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

Burnett & Rossi, Chem Biol, 2012
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

efficient mechanism for inserting their 'foreign' genetic material into the host cell and for persuading the host cell to express that genetic material
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

Plasmid

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

Recombinant DNA
Transformation of bacteria

double-stranded recombinant plasmid DNA introduced into bacterial cell

bacterial cell

cell culture produces hundreds of millions of new bacteria

many copies of purified plasmid isolated from lysed bacterial cells

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, it 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 of 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
## Non-viral vectors - summary

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<th>Vector</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<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 in 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

LTR - long terminal repeats

Retroviral vector
Lentiviral vectors – a special type of retroviral vectors

- developed from highly pathogenic HIV virus
- for non-dividing cells
- integrate into genome
- widespread clinical application after rigorous safety testing
Adenoviral vectors

- Adenoviruses - DNA paroviruses 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
Adeno-associated vectors (AAVs)

- Linear single-stranded DNA paroviruses 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
Construction of AAV vectors

system with helper adenoviral

Helper-free system
no adenovirus required

Co-transfect AAV-293 cells with:
Recombinant pAAV Vector
pAAV-RC
pHelper

Produce AAV Particles in AAV-293 cells
## Viral vectors - summary

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<th>Vector</th>
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<th>Disadvantages</th>
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<tr>
<td>Viral vectors</td>
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<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>
</tr>
<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>cells, inserts up to 30 kb</td>
<td>gene expression, will target non-dividing cell</td>
<td></td>
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<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>cell</td>
<td>gene expression</td>
<td>insert size (&lt; 4.5 kb)</td>
</tr>
<tr>
<td>Herpes simplex virus (HSV)</td>
<td>Readily enters CNS, inserts up to 30 kb</td>
<td>Risk of virulent reversion</td>
</tr>
</tbody>
</table>

### Integrating
- retroviral
- lentiviral
- AAV

### Non-integrating
- adenoviral
- HSV

**Integration depends on:**
- *LTR sequences and integrase (retroviruses)*
- *ITR sequences and rep proteins (AAV)*
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
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.
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**
Cell-specific promoters

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, Tie-2
- **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.

- **tTS cassette**: promoter, tetR, Kid-1
- **rtTA cassette**: promoter, rtetR, VP16

**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)

**Expression**
- When doxycycline/Tet is present
- Protein expression

Jazwa et al., Gene 2013
Mechanisms of blood vessels formation – involvement of heme oxygenase – 1

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

**vasculogenesis**

Formation of new blood vessels from pre-existing ones

**angiogenesis**

- Formation of new blood vessels *de novo* from vascular progenitor cells
- Formation of new blood vessels from pre-existing ones

M. Tertil, praca doktorska 2013
Jujo et al., 2008, Käßmeyer et al., 2009
Murine model of angiogenesis – hind limb ischemia

After Hiroshi Niiyama, Ngan Huang, Mark Rollins, John Cooke, Stanford University

Laser Doppler Perfusion Imager (LDPI) System

---

After: Hiroshi Niiyama, Ngan Huang, Mark Rollins, John Cooke, Stanford University

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the sites of ligation of femoral artery

Medial femoral artery
Inguinal ligament
Femoral nerve
Femoral vein
Proximal ligation site
Distal ligation site
Superficial epigastric artery
Popliteal artery
Saphenous artery

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

HIF1α proteasomal degradation

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

- ↓ H$_2$O$_2$-induced cell death of endothelial cells
- ↑ proliferation of endothelial cells
- ↓ post-ischemic inflammation
- ↓ post-ischemic muscle cell death
- ↑ post-ischemic blood flow
- ↑ regenerative potential of muscles

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

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

MicroRNA targeted vectors may allow for 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 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
    - 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

- 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-regulated vectors for expression of Cre may be used

- zinc finger nucleases**
  - random insertions/deletions due to cellular repair mechanisms (NHEJ) after ZFN action
  - homologous recombination-mediated correction of gene (repair donor sequence co-transfected with vectors expressing ZFN)

**DNA sequence-specific binding

Jazwa et al. Gene 2013
Gene therapy clinical trials

Up to date, cancer is by far the most common disease treated by gene therapy.
Currently, more than 1800 approved gene therapy clinical trials worldwide have been conducted or are still ongoing.

Adenoviral vectors, retroviral vectors and naked plasmid have been the most commonly used gene transfer vectors in clinical trials.
### Gene therapy successes

#### 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

- 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 clinical benefit.

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

(Bainbridge et al., 2008; Cideciyan et al., 2008; Maguire et al., 2008)
AAV vectors for the treatment of inherited blindness

Biological activity resulting from localized gene therapy in three patients with inherited blindness

Retinal loci demonstrating significant change (stars) in light sensitivity at 1,2 and 3 months after treatment compared with before treatment.

All significant changes were increases in sensitivity and correspond to regions of study eyes that received gene therapy. Control eyes of the three patients did not show significant changes.
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
Gene therapy strategies for HIV/AIDS

gene therapy approaches that 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 CD34+ HSCs and CD4+ T cells</td>
<td>Lentiviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>Fusion inhibitor C46 (env protein)</td>
<td>Autologous CD4+ T cells</td>
<td>Retroviral vector</td>
<td>I-II, Ongoing</td>
</tr>
<tr>
<td>Fusion inhibitor C46 (env protein)</td>
<td>Autologous CD4+ T cells</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 CD8+ T cells</td>
<td>Lentiviral 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>
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.

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)
Mechanism of action of Oncorine

- a conditionally replicative adenovirus
- contains a deletion in E1B 55K region, which restricts the virus to bind and inactivate wild-type p53 protein
- When E1B 55K activity is removed, the replication in normal cells is blocked, allowing only replication in p53-deficient cells
- In malignant cells the viral proliferation leads to oncolysis, which is used as a cancer therapy to treat solid tumours

A deletion in E1B region restricts the virus to bind and inactivate wild-type p53 protein resulting in the replication in p53-deficient cells only.
- 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