# Gene therapy

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## Spis treści

# Definition

**Gene therapy** is a medical process where the wildtype (normal) version of a gene is introduced into a patient's cells to treat the disease caused by the mutant form of the gene, which failed to function properly.

Types of gene therapy:

- ***In vivo*** gene therapy
- ***Ex vivo*** gene therapy

# Vectors

Vectors are vehicles that can accurately target the therapeutic gene into the correct cells in the patient's body.

- viral or nonviral

## Adenoviral vectors

- linear ds DNA
- non-integrating (time-limited expression)
- capacity: 8kb (>20kb for the last generation)
- infect replicating and differentiated cells
- immunogenic
- can be used as oncolytic viruses for the treatment of cancer (e.g. Onyx15)

## Retroviral vectors

- integration with host genome ➔ lifetime expression
- capacity: 8kb
- only infect dividing cells (exception: lentivirus)
- random integration ➔ can cause oncogenesis
- immunogenic

## AAV vectors

- adeno-associated virus
- very small capacity (4 – 4.5kb)
- defective replication
- 12 serotypes, showing tissue-specific tropism
- no pathogenicity; low immunogenicity
- Transgene expression – sustained for ~2 years
- ineffective, nonspecific genome integration without Rep protein (most of vector DNA resides as episomes)

## Liposomes

- hollow spheres surrounded with membranes made up of phospholipids
- can be used to transport therapeutic gene

## Gendicine

- p53 protein
    - Transciption factor, responsible for cel life cycle regulation, DNA repair and induction of apoptosis

- p53 mutation is the most frequent change observed in neoplasms
- Adenoviral vector carrying a functional copy of p53 gene

**Table 3.** Comparison of Protocol Efficiency

| Protocol | N | 4 Wks Treatment CR | PR | SD | PD | N | 8 Wks Treatment CR | PR | SD | PD | N | 4 Wks Follow-Up CR | PR | SD | PD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GTRT | 63 | 8% | 65% | 27% | 0% | 62 | 45% | 47% | 8% | 0% | 56 | 64% | 29% | 7% | 0% |
| RT | 72 | 0% | 40% | 57% | 3% | 71 | 8% | 62% | 28% | 0% | 63 | 19% | 60% | 21% | 0% |

Comparison of GTRT group with RT group, p <0.01 in each comparison
CR: complete response, PR: partial response, SD: stabilized disease PD: progressive disease

- Available in China since 2003; treatment cost: 3800 yuan (~540$) / dose

### Oncorine (Onyx-015)

- An experimental **oncolytic virus** (modified adenovirus)
- Adenovirus without E1B gene ➔ unable to inactivate the p53 protein
  - Can replicate effectively only in cells with nonfunctional p53 (cancer cells)
- Clinical trials: greater response rate, lesser probability of progression in patients where Onyx-015 was introduced to the therapy

## Examples of gene therapy

### SCID

- severe combined immunodeficiency disease
- disease variant caused by mutation in **adenosine deaminase (ADA)**
  - **ADA deficiency** leads to accumulation of **toxic levels** of intracellular **purine metabolites** and thus impairment of T- and B-cells
  - loss of T- and B-cells functions presents clinically as **inability to recover from even a mild course of infection**
- ex vivo treatment
  - Lymphocyte cells were removed from patients and treated with retroviral vectors **carrying wildtype ADA gene**
  - modified lymphocytes were then returned to the patient's body
- Improved procedure:
  - hematopoietic stem cells instead of lymphocytes – isolated and transduced with retroviral vectors
  - before engrafting the cells back - low-intensity, nonmyeloablative conditioning regimen (busulfan 2 mg per kg per day), to provide an initial developmental advantage to transduced HSCs and create space in the bone marrow
- Direct result of clinical trials: Strimvelis
  - gene therapy for SCID (ADA deficiency) available in Europe since May 2016
  - $648 000

### B-ALL

- Acute B Lymphoblastic Leukemia
- Development of large numbers of immature lymphocytes
- The most common leukemia found in children

- CAR: Chimeric Antigen Receptor
    - Recognizes CD19 antigen, present on B-lymphocytes surface
    - Treatment of B-cell precursor acute lymphoblastic leukemia
- T-lymphocytes – isolated from patient, transduced with vectors carrying CAR
    - can recognize and destroy B-cells
- 30.08.2017: KYMRIAH (tisagenlecleucel)
    - gene therapy registered by FDA
    - $475000

## Cystic fibrosis

- Occurs when a person inherits two mutant **cystic fibrosis transmembrane conductance regulator** (CFTR) alleles
    - nonfunctional chloride ion channel
    - chloride ions accumulated inside the cells
    - most common mutation: 508Phe deletion
- Phe508Del
    - defective protein processing, leading to considerable endoplasmic reticulum (ER) retention and premature degradation
    - impaired trafficking of mutated protein to the cel Surface
    - only partial channel function and a highly decreased half-life
    - Responsible for ~70% of CF cases
- Organ with most severe damage: lungs, pancreas
- ivacaftor
    - treatment option, available from 2012, for patients with specific CFTR mutation, resulting in constantly closed CFTR channel
    - The drug enables the opening of the ion channel
    - Annual cost of treatment: $300000
- Elexacaftor–Tezacaftor–Ivacaftor
    - Ivacaftor combined with new generation drugs
        - Tezacaftor: CFTR potentiator; corrects the functionality of F508Del protein
        - Elexacaftor: increases the effectiveness of mutgated CFTR trafficking to the cell surface
- Gene therapy:
    - wildtype CFTR in liposome vector
    - placebo and treated groups: inhalations once per month
    - stabilized FEV1 in the treatment group
    - no significant difference in treatment-attributable adverse events between groups

## Duchenne muscular dystrophy

- The **most prevalent** type of **muscular dystrophy** disease in humans
    - 13-33 per 100 000 births (boys only)
- causes **rapid degeneration of muscle tissues** early in life
- the cause: mutations in dystrophin gene
    - The **largest known human gene** (more than **2.6 million bases**), contains **97 exon sequences**
    - **nonsense** or **frameshift** mutations, leading to complete lack of dystrophin, result in Duchenne muscular dystrophy
    - missense mutations are responsible for **Becker** muscular dystrophy (milder form)

- Ataluren
    - A drug for ~15% of patients, carrying nonsense mutation
    - The drug binds to ribosome enabling it to continue translation despite the premature STOP codon
    - Clinical trial confirmed the positive effect in boys with DMD (significant increase in 6-minute walk distance)
- Gene therapy
    - very long coding sequence (~11 kb) – too much for AAV, which could be a perfect vector (serotypes with muscle tropism)
    - micro- / mini- dystrophin: much shorter, functional gene
    - preclinical study on dogs resulted in very promising outcomes
- Phase 1b clinical trial (Pfizer Inc.)
    - AAV9 carrying human minidystrophin (PF-06939926)
    - One-time intravenous administration (high or low dose)
    - Preliminary report on 9 patients available since may 2020
    - 9 patients, aged 6-12
    - Significant increase of dystrophin concentration in muscles
    - Improved North Star Ambulatory Assessment score for patients undergoing the therapy, compared to untreated controls
    - Most common adverse effects: vomiting, nausea, decreased appetite, and pyrexia
    - 3 cases of severe adverse events:
        - persistent vomiting resulting in dehydration
        - acute kidney injury with atypical hemolytic uremic syndrome (aHUS)-like complement activation
        - thrombocytopenia with aHUS-like complement activation
    - All of them were fully resolved and at their last clinic visits, all patients were doing well

# Dental applications

## Periodontal regeneration

### BMP7

- a part of a family of proteins that regulates cartilage and bone formation.
- significant role in tooth development and periodontal repair
- animal study: application of BMP-7 in adenoviral vector significantly increased the bone regeneration rate

### RUNX2

- Runt-related transcription factor 2
- Transcription factor necessary for osteoblast differentiation
- the use of adenoviral vector carrying RUNX-2 gene resulted in increased regeneration rate and better mineralization of bones

### PDGF

- FDA-approved for the treatment of neurotrophic diabetic ulcers and for promoting bone repair of periodontal osseous defects
- PDGF gene transfer stimulates gingival fibroblast and cementoblast mitogenesis and proliferation to a greater extent than continuous PDGF *administration in vitro*

- fibroblasts were transduced with adenovirus encoding PDGF-A, PDGF-B or GFP genes
- PDGF-B gene transfer stimulated potent (>4-fold) increases in cell repopulation and defect fill above that of PDGF-A and corresponding controls

### Radiation-induced salivary hypofunction

- Head and neck cancer patients after radiation ➔ xerostomy (damage of salivary glands)
- 2012: first clinical trial of AQP1 (aquaporine ➔ a channel for water molecules) gene therapy for the treatment of radiotherapy-induced xerostomy
- AQP1 in adenoviral vector
- Improvement observed in half of patients

### DNA vaccines

- Rats' salivary glands transformed with plasmid carrying a gene encoding fimbria protein of *Porphyromonas gingivalis*
    - Synthesis of salivary antibodies and cytotoxic lymphocytes against the antigen
- Plasmid with PAC gene from *Streptococcus mutans* (main bacterial antigene anchored to the cell wall)
    - Induction of anticaries immune response in rats

### Orthodontic Tooth Movement

- RANK: Receptor activator of nuclear factor κ B
- RANKL: ligand for RANK
- Osteoclastic precursors express on their surface RANK; binding RANKL initiates their fusion into multinucleated giant cells
- Osteoblasts release OPG (osteoprotegerin), a soluble receptor which also binds RANKL (competing woth RANK), therefore inhibiting osteoclastogenesis
- Animal study results
    - Local RANKL gene transfer to periodontal tissue resulted in accelerated orthodontic tooth movement by approximately 150% after 21 days, without evoking any systemic effects, significantly reducing the time of treatment
    - In contrast to RANKL, local OPG gene transfer inhibited tooth movement by about 50%

## Silencing of genes

For some disorders, the problem lies in expression of harmful gene

- dominant disease-causing allele
- proto-oncogenes
- VEGF in tumors

How to turn off their expression?

### RNA interference – definition

RNA interference (RNAi) is a natural process of degradation or expression inhibition of target transcript by specific short RNA molecules (siRNA or miRNA).

- by designing proper siRNAs, we can shut down expression of harmful genes

## Periodontitis – CtsK

- Periodontitis: a chronic inflammatory disease that damages the soft tissue and destroys the alveolar bone that supports the teeth, which ultimately lead to tooth loss
    - *Porphyromonas gingivalis* is one of the most prominent pathogens highly associated with periodontitis
    - the inflammatory spectrum associated with the host response in the gingival tissues trigger enhanced bone resorption by osteoclasts
- Cathepsin K (Ctsk)
    - a lysosomal cysteine protease
    - strongly expressed by osteoclasts
    - Degrades protein components of bone matrix ➔ critical for osteoclastic bone resorption
- Gene therapy:
    - Mouse model of periodontits
    - shRNA was designed, matching Ctsk mRNA (to silence its expression)
    - AAV carrying Ctsk shRNA was administrated locally into the periodontal tissues *in vivo*
- Results:
    - Silencing of cathepsin K expression drastically protected mice (>80%) from *P. gingivalis*-induced bone resorption by osteoclasts
    - Also, the therapy significantly reduced inflammation by impacting the expression of many inflammatory cytokines as well as T cell and dendritic cell numbers in periodontal lesions
- As a result, AAV-sh-Ctsk administration can efficiently protect against periodontal tissue damage and alveolar bone loss

# CRISPR

## Definition

- Clustered Regularly Interspaced Short Palindromic Repeats
- Prokaryote equivalent for RNAi
    - Provides protection from viral DNA
    - bacterial Cas9 nuclease cuts target DNA in a place indicated by specific RNA molecule (gRNA)

## Potential application

- Changing gRNA sequence we can program a target seqence in our genome to be cut
- Almost every fragment of our genome can be targeted by Cas9
- Easy *in vitro* application
- Very high specifity (The cut is applied only in the place targeted by gRNA)  High transformation efficiency (~70%)
- Many targets can be cut in a single experiment
- CRISPR allows for „genome editing” – there is no need of sophisticated vector systems that would provide sustained expression of gRNA and Cas9
    - Only temporary expression is required

## CRISPR-mediated gene knockout

- The cut is repaired in a proces called Non-Homologous End Joining (NHEJ)
- Error-prone ➔ introducing mutation to target site

## CRISPR for expression activation/inactivation

- dCas9: „dead Cas9"
- DNA binding, no nuclease activity
- Fusing dCas9 with transcription repressors/activators, targeting promoter sites

## Repair of target sequence with CRISPR

- HDR (Homology Directed Repair), using a DNA template with correct, nonmutated gene sequence

## CRISPR – DMD

- 60% of mutations responsible for DMD – exons 45-55
- Using CRISPR to cut out these exons
- As a result – almost fully functional shorter dystrophine; mild symptoms – after 60 years of age

## CRISPR – retinitis pigmentosa

- Loss of vision due to progressive loss of rod photoreceptor cells in the back of the eye
- Autosomal dominant disorder
    - Gain of function mutation in rhodopsin gene (*Rho*$^{S334}$)
- Preparation of gRNA targeting mutated dominant allele
    - Introduction of mutations after DNA is cleaved and repaired ➔ conversion to recessive allele
    - Most patients – heterozygotes, having a wildtype allele

## CRISPR – Huntington's disease

- caused by a **triplet repeat mutation** in the coding region of the **huntingtin** gene (expansion of CAG triplet, encoding glutamin)
    - Autosomal dominant disorder
- **Mutant** huntingtin proteins contain regions where the amino acid **glutamine can be repeated hundreds of times** (the treshold is **>35 repeats**)
    - Such mutated protein cannot be metabolised ➔ accumulation of aggregates in the brain
- CRISPR/Cas9 – cutting-out the fragment encoding poly-glutamine chain
- animal model (mouse)
    - 140 Gln repetitions in HTT gene
    - 9-10 month old mouse have huntigtin aggregates in the brain
    - Two AAV vectors (injected to striatum)
        - 2 gRNAs (T1, T3) under U6 promoter
        - Cas9 under CMV promoter
    - Significant reduction of huntigtin content in the cells
    - Significant imporvement in motor functions