Examples of clinical applications
Gene therapy

*use of nucleic acids as drugs*

**Therapeutic gene**  
**Vector**  
**Patient**

Expression of therapeutic gene; therapeutic effects
Gene therapy

Enhancing
- enhancement of gene expression
  - Acquired diseases

Substituting
- delivery of the missing gene
  - Inherited diseases

Suppressive
- inhibition of gene expression
  - Acquired diseases
Therapeutic nucleic acids

DNA
- Genes (delivered by DNA vectors)
  - Antisense oligonucleotides
  - DNA decoys
- Non-coding sequences

RNA
- Genes (delivered by RNA vectors)
  - Ribozymes
  - siRNA
- Non-coding sequences
Vectors

Non-viral/plasmids

- „naked“ DNA
- Lipoplexes
- Viroplexes (lipoplexes enhanced in proteins from viral capsids)

- Complexes With chemical vehicles

viral

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

- RNA
- DNA
The four barriers of successful gene therapy

Kay M, Nature Rev Genetics, 2011
Naked plasmid, containing lacZ gene has been injected into the leg muscle of a mouse.
VEGF-A is a major angiogenic growth factor. It acts on endothelial cells, being produced by numerous cell types, including vascular smooth muscle cells (VSMC), fibroblasts or tumor cells.
Scheme of experiment

- Concentration of VEGF protein in the treated muscle
- Number of capillaries in the treated muscle
- Blood flow in the treated muscle

Plasmid encoding VEGF

pSG5-VEGF

2 weeks
Effect of plasmid encoding VEGF on number of capillaries in the treated muscle

Control leg

Ischemic leg injected with β-galactosidase

Ischemic leg injected with pVEGF165

(14 days after injection)
Effect of injection of pVEGF_{165} plasmid on tissue perfusion in the rabbit muscle

Before ligation

Immediately after ligation

14 days after ligation

Peripheral atherogenesis

- Peripheral arterial disease results from formation of atherosclerotic plaques in peripheral arteries, mostly in the legs. This leads to narrowing the vessel lumen, decreased blood flow and tissue ischemia.
- The typical symptom is strong pain (mostly during walking, but also in resting legs) and intermittent claudication.
- In the most severe cases it may lead even to toe or foot necrosis and necessity of amputation.

- Treatment relies on application of antithrombotic agents, lowering cholesterol and triglycerides.
- Surgical treatment involves athorectomy, angioplasty, and bypassing.
First attempt of VEGF to the ischemic leg – 1996

- **Patient:** 71-year old woman with severe leg ischemia
- **Treatment:** 2 mg of plasmid DNA coding for vascular endothelial growth factor (VEGF) delivered in hydrogel using a balloon catheter.
- **Effects:** increased number of blood vessels and improved blood flow in the treated leg.
- **Side-effects:** development of angiomas one week after gene delivery (one of them was surgically removed, two disappeared), local edema (cured pharmacologically)

*Improvement was not enough to save the leg.*
*Amputation was performed 5 months after gene delivery.*
Angiography:

Evidence of formation of new collateral blood vessels in ischemic leg after delivery of 4 mg of plasmid VEGF.
Figure 4. One of the first persons treated with an angiogenesis-promoting growth factor gene, a 33-year-old woman with a non-healing wound on the medial aspect of the calf and ischemic necrosis of the great toe (top), was able to successfully avoid amputation. Ingrowth of granulation tissue in the calf wound and reduction of the toe lesion were observed within a few weeks of initiating therapy (middle). At three months, after placement of a split-thickness skin graft, the calf wound had healed, and the toe necrosis had completely resolved (bottom). (From Baumgartner et al, 1998)
KAT trial – improvement only after adenoviral delivery

Panels d and e depict 54-year old male patient with a significant stenosis in left anterior descending artery before (d) and 6 months after (e) gene transfer. Myocardial perfusion during adenosine infusion: Arrows indicate perfusion defect area.
<table>
<thead>
<tr>
<th>Transgene and vector</th>
<th>Route and indication</th>
<th>References</th>
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<tbody>
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<td>Makinen et al. (2002)&lt;sup&gt;16&lt;/sup&gt;</td>
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<td>VEGF&lt;sub&gt;121&lt;/sub&gt;</td>
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<td>Adenovirus</td>
<td>Intramuscular for PAD</td>
<td>Rajagopalan et al. (2002)&lt;sup&gt;17&lt;/sup&gt; and Rajagopalan et al. (2003)&lt;sup&gt;18&lt;/sup&gt;</td>
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<td>Adenovirus</td>
<td>Intramyocardial for CAD</td>
<td>Rosengart et al. (1999)&lt;sup&gt;35&lt;/sup&gt;</td>
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<td>VEGF-2</td>
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<td>Comerota et al. (2002)&lt;sup&gt;19&lt;/sup&gt;</td>
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<td>Grines et al. (2002)&lt;sup&gt;37&lt;/sup&gt; and Grines et al. (2003)&lt;sup&gt;38&lt;/sup&gt;</td>
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<td>HGF</td>
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<tr>
<td>Plasmid</td>
<td>Intramuscular for PAD</td>
<td>Morishita et al. (2004)&lt;sup&gt;20&lt;/sup&gt;</td>
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CAD, coronary artery disease; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; PAD, peripheral arterial disease; TO, thromboangiitis obliterans; VEGF, vascular endothelial growth factor.
Diabetic retinopathy
Vascular endothelial growth factor-A (VEGF)

VEGF-A is a major angiogenic growth factor. It acts on endothelial cells, being produced by numerous cell types, including vascular smooth muscle cells (VSMC), fibroblasts or tumor cells.
Macugen (aptamer blocking VEGF) – first antiangiogenic drug approved for treatment of ophthalmological diseases.

Inhibition of VEGF binding to VEGF receptors in cells treated with macugen
V.I.S.I.O.N. trial results

Early and Sustained Treatment Benefit

Weeks

50% Benefit

*P<0.01
Gene therapy for cancer
Why there is a need for cancer gene therapy
- 5-years survival rates of patients with different cancer
Natural oncolytic viruses

Ras protein

EGFR-I

Growth factors eg. EGF

EGFR

EGFR-I sensitivity

Mutations present

EGFR-I

Gefitinib

Erlotinib

PI3K

AKT

RAF

MEK

MAPK

Cell proliferation

Antiviral response

PKR
Ras proteins in cancer cells

Approximately 60% of cancer cells have overactive Ras proteins:

- Mutations leading to formation of constitutively active proteins (20-30%)
- Mutations in Ras-activating proteins, leading to constitutive activation of Ras
- Mutations in oncosuppressor genes, attenuating inhibition of Ras activity
Natural oncolytic virus

Reoviruses – replicate exclusively in cells with overactive Ras proteins

Ovary carcinoma cells

Before

After

Reovirus replicates in Ras-activated cancer cells, causing virus-mediated cell death

Tumour antigens generated by viral oncolysis may educate the immune system to recognize and kill tumour cells
Therapy with oncoviruses - Reolysin

Virus attaches to tumour cell surface, then enters cell.

After envelope is removed, replication of viral genome ensues, followed by viral protein synthesis.

Accumulation of virions is followed by host-cell lysis; new viral particles are released to infect nearby tumour cells.

New virions are encapsulated as host cellular protein synthesis is shut down.
Viruses used in anticancer therapy

Viruses modified to destroy mostly cancer cells, but not healthy cells

Viruses modified to deliver "killing genes" into the cancer cells

Viruses modified to deliver genes into the immune cells and make them more effective in killing cancer cells

Wild type viral genome

Modified viral genome
Melanoma
melanoma cell

Tc - activated
Lymphocytes Tc of one patient were exceptionally effective in killing melanoma cell. Their receptors were highly efficient in recognizing MART-1 antigens on melanoma cells.

several years ago....

Harvesting of tumor infiltrating lymphocytes

Selection of lymphocytes most effective in tumor recognizing and killing

Injection of multiplied cells to the same patient

In some patients disease was almost completely cured

Dudley et al. Science 2002

- Lymphocytes Tc of one patient were exceptionally effective in killing melanoma cell.
- Their receptors were highly efficient in recognizing MART-1 antigens on melanoma cells.
Retroviral vectors

- Retroviral vectors (based on RNA viruses):
  * one of the most often used vectors
  * introduce transgene into host genome - are perfect for developing stably transfected cell lines
  * do not interfere with cell functions
  * can infect dividing cells only (with exception of lentivirus)
  * capacity: ~7 kb
  * suitable for in vitro applications – are easily destroyed by complement cascade
then....

1. Receptor consists of two proteins ($\alpha$ i $\beta$), encoded by separate genes – both were cloned from lymphocytes of the cured patient

2. Genes were introduced to the retroviral vector

Genome of retroviral vector TcR antiMART-1

Retroviral vector TcR antiMART-1
Scheme of gene therapy with TcR antiMART-1 gene delivered by means of retrovirual vector
...2 patients do not have any symptoms of melanoma today (84 months after therapy)...

Matastatic melanoma in the liver
Adenoviral vectors

- Adenoviral vectors (based on DNA viruses):
  * one of most often used, universal vectors, which can be produced at high titers
  * very efficiently transduce many different cell lines, both proliferating and not proliferating
  * remain as episomal element, giving very strong but short-term expression
  * capacity: ~9 kb
  * genes of E1 region are necessary for expression of all other viral genes

[Diagram of adenoviral vector structure]
Adenoviruses

- Adenoviruses cause common colds, conjunctivitis, and diarrhea.
- Can transduce all cell types.
- Genome consists of double DNA strain.
  The crucial genes are gene of region E1.
- E1A protein is necessary for expression of remaining viral genes.
- E1A is necessary for effective viral replication.
Ad with E1A gene under control of a cellular promoter that is preferentially active in tumor cells; eg. hTERT
Adenoviruses

- Adenoviruses cause common colds, conjunctivitis, and diarrhea.
- Can transduce all cell types.
- Genome consists of double DNA strain.
- The crucial genes are gene of region E1.
- E1B-55 protein blocks activity of p53.
Oncolytic virus

A virus that preferentially infects and lyses cancer cells
P53 protein – guardian of genome

P53 proteins is very often inactive in cancer cells
Modified adenoviral vector: ONYX-015

Adenoviruses lacking E1B gene cannot replicate in cells possessing active p53 (healthy), but only with inactive p53 (neoplastic).

Therefore they can specifically destroy cancer cells.
Healthy cells

Virus without E1B

Viral replication

P53+

No viral replication

P53−

Viral replication

Cytolysis + viral spread

Cells survive

Tumor cells
Prostate cancer cells cultured in vitro and infected with E1B-deficient adnoviruses
Effects of treatments of patients suffering from head and neck cancer with ONYX-015

Chemotherapy + virus

Chemotherapy

N=11, P<0.006
Oncorine – accepted for clinical use in China

* In November 2005, SFDA (China) approved H101 virus (commercially sold as Oncorine)

* It is a genetically-modified type-five adenovirus which can selectively replicate inside tumour cells with dysfunctional p53 genes, killing them and stopping the cancer's spread.

* This virus is an improved ONYX-015 virus lacking B55 protein.
Viral vectors in tumor therapy

- Virus attaches to and infects tumor cell
- Foreign gene(s) of interest expressed
- No new viruses are produced
Gendicine – accepted for clinical use in China

*Advexin* (Introgen, USA)

Adenoviral vector with a correct p53 gene
Already at phase III of clinical trials

- **Gendicine** is an adenoviral vector coding for p53.
- In October 2003, China's State Food and Drug Administration (SFDA) approved **Gendicine**, after the medicine showed some promising results in tumour regression among 99 head and neck squamous cell carcinoma patients.
Suicide gene therapy

**Thymidine kinase (Tk)** – enzyme derived from herper simplex virus (HSV) catalysing phosphorylation of ganciclovir. Resulting ganciclovir triphosphate is a potent inhibitor of DNA polymerase, leading to cell death.

**Ganciclovir** – analog of guanosine used in patients infected with cytomegalovirus (CMV)

**Procedure:**
Cells are transduced with Tk gene. Then cells are treated with ganciclovir. Tk convert the prodrug in the toxic drug, leading to the death of cells.
Glioma – brain tumor

• Glioblastoma multiforme (GMB) – infiltrative, most malignant and incurable tumor arising from glial cells in the brain

• Mean survival time of patients after diagnosis is ~6 months, despite surgery, radio- and chemotherapy.

• Tumor does not form metastasis, but leads to death because of increased volume and destruction of brain tissue.

• Develops in adults (mean age of diagnosis – 45 years)
Suicide gene therapy in GBM

Procedure:

1. Most tumor tissue is removed during surgery.

2. Viral vector harboring Tk is injected into wound bed after tumor resection.

3. Vectors delivers Tk gene into tumor cells.

4. Patient receives ganciclovir for several days, which is converted by Tk to the toxic drug, causing tumor cells’ death.
Suicide gene therapy of glioblastoma

Figure 2: Brains of rats injected in with 9L cells of gliosarcoma. Left - “HSV/tk ganciclovir Group” Only an empty cavity is observed at the site where before there was the tumour. Right - “Control Group” - The growing tumour occupies almost all the left frontal lobe of the rat.

Department of Neurosurgery, University of Kuopio, A.I. Virtanen Institute, Finland.

Malignant glioma is a devastating brain tumor with no effective treatment. This randomised, controlled study involved 36 patients with operable primary or recurrent malignant glioma. Seventeen patients were randomized to receive AdvHSV-tk gene therapy (3 x 10^{10} pfu) by local injection into the wound bed after tumor resection, followed by intravenous ganciclovir (GCV), 5 mg/kg twice daily for 14 days. The control group of 19 patients received standard care consisting of radical excision followed by radiotherapy in those patients with primary tumors. The primary end-point was survival as defined by death or surgery for recurrence. Secondary end-points were all-cause mortality and tumour progression as determined by MRI. Overall safety and quality of life were also assessed. Findings were also compared with historical controls (n = 36) from the same unit over 2 years preceding the study. AdvHSV-tk treatment produced a clinically and statistically significant increase in mean survival from 39.0 +/- 19.7 (SD) to 70.6 +/- 52.9 weeks (P = 0.0095, log-rank regression vs. randomized controls). The median survival time increased from 37.7 to 62.4 weeks. Six patients had increased anti-adenovirus antibody titers, without adverse effects. The treatment was well tolerated. It is concluded that AdvHSV-tk gene therapy with GCV is a potential new treatment for operable primary or recurrent high-grade glioma.
AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma.

*a randomised, controlled study, 36 patients*

III phase clinical trial of Cerepro: Mean survival time – 15.5 months in treated group versus 9 months in control

*Immonen A, Mol Ther. 2004*
Suicide gene therapy of glioblastoma

Before treatment

Two years and two months after vector injection

(survival: 29 months after treatment)
Cerepro™ has been granted Orphan Drug Status by the European Committee for Orphan Medicinal Products and by the Office of Orphan Products Development, FDA

London, UK – 18 January 2008 - Ark Therapeutics announces today that, following a recent independent Data and Safety Monitoring Board (“DSMB”) review of its Phase III trial for Cerepro® (Study 904), the Company will continue to follow patients in the study until the end of June. At this point the Company will have a sufficiently full data set to provide reliable statistical evaluations of the clinical effects of Cerepro®. Cerepro® is Ark’s lead product for the treatment of high grade glioma (malignant brain tumour).

250 patients are randomised in a 1:1 ratio either to standard care alone or to standard care plus Cerepro® treatment and patients are blinded to the point of treatment allocation. The multi-centre study is being conducted in Europe and Israel.

Trials completed to date have shown that Cerepro® treatment produces an average extension of 6.5 months of life, giving around 15.5 months survival, whereas controls survived on average for around 9 months.

Last year the application was rejected by EMEA
Phases of Gene Therapy Clinical Trials

- Phase I 60.8% (n=1035)
- Phase I/II 18.3% (n=312)
- Phase II 16.4% (n=279)
- Phase II/III 0.8% (n=14)
- Phase III 3.5% (n=59)
- Phase IV 0.1% (n=2)
- Single subject 0.1% (n=2)
Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 64.5% (n=1098)
- Cardiovascular diseases 8.5% (n=144)
- Monogenic diseases 8.3% (n=141)
- Infectious diseases 8% (n=137)
- Neurological diseases 1.8% (n=30)
- Ocular diseases 1.2% (n=20)
- Other diseases 2.5% (n=43)
- Gene marking 2.9% (n=50)
- Healthy volunteers 2.3% (n=40)
Vectors Used in Gene Therapy Clinical Trials

- Adenovirus 24.1% (n=410)
- Retrovirus 20.8% (n=354)
- Naked/Plasmid DNA 18.7% (n=319)
- Vaccinia virus 8% (n=137)
- Lipofection 6.4% (n=109)
- Poxvirus 5.5% (n=94)
- Adeno-associated virus 4.8% (n=81)
- Herpes simplex virus 3.3% (n=57)
- Lentivirus 2.2% (n=38)
- Other categories 5.2% (n=89)
- Unknown 3.2% (n=55)
Gene therapy is successful in the treatment of diseases.

<table>
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<tr>
<th>Disorder</th>
<th>Disease type</th>
<th>Patients benefiting</th>
<th>First publication</th>
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<td>X-SCID</td>
<td>Immunodeficiency</td>
<td>17/20</td>
<td>2000</td>
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<tr>
<td>ADA-SCID</td>
<td>Immunodeficiency</td>
<td>26/37</td>
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<td>Adrenoleukodystrophy</td>
<td>Neurologic</td>
<td>2/4*</td>
<td>2009</td>
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<td>Leber’s congenital amaurosis</td>
<td>Blindness</td>
<td>28/30</td>
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<td>Wiskott-Aldrich syndrome</td>
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<td>8/10</td>
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<td>Hemophilia</td>
<td>Coagulation</td>
<td>6/6</td>
<td>2011?</td>
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*Includes a patient treated too recently to see benefit
Next lecture – 15th May

When exam?

19 or 26th June

Only one term!