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

Suppresive
- inhibition of gene expression
- Acquired diseases
Phases of Gene Therapy Clinical Trials

- Phase I 60.3% (n=952)
- Phase I/II 18.9% (n=299)
- Phase II 16.3% (n=258)
- Phase II/III 0.8% (n=13)
- Phase III 3.4% (n=53)
- Phase IV 0.1% (n=2)
- Single subject 0.1% (n=2)
Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 64.5% (n=1019)
- Cardiovascular diseases 8.7% (n=138)
- Monogenic diseases 7.9% (n=125)
- Infectious diseases 8% (n=127)
- Neurological diseases 1.9% (n=30)
- Ocular diseases 1.1% (n=18)
- Other diseases 2.2% (n=33)
- Gene marking 3.3% (n=50)
- Healthy volunteers 2.3% (n=37)
Vectors Used in Gene Therapy Clinical Trials

- Adenovirus 23.9% (n=387)
- Retrovirus 20.8% (n=336)
- Naked/Plasmid DNA 17.7% (n=287)
- Vaccinia virus 8% (n=129)
- Lipofection 6.7% (n=109)
- Poxvirus 5.7% (n=93)
- Adeno-associated virus 4.4% (n=71)
- Herpes simplex virus 3.4% (n=55)
- RNA transfer 1.4% (n=23)
- Other categories 4.8% (n=77)
- Unknown 3.2% (n=51)
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
Expression of human VEGF protein in the rabbit muscle

VEGF protein [pg/mg]

β-galactosidase  pSG5-VEGF in fibrin  pSG5-VEGF in PBS

Detection threshold
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

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 cholestrol and triglicerydes.
- Surgical treatment involves athorectomy, angioplasty, and bypassing.
First attampt 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 hydrogen 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.*
Clinical trial of gene therapy in treatment of peripheral arterial disease

Double-blind, placebo controlled trial. 54 patients received VEGF cDNA in plasmid injected with liposomes or in adenoviral vector. The carriers were delivered into arteries using bloon catheter.

Results:

– No side effects of the therapy (assessed after three months).
– Increased number of blood vessels in patients treated with therapeutic cDNA using adenoviral vectors.
– Better clinical outcome (and better subjective feeling of patients) detected both in VEGF and placebo treated patients....
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)

Plasmid VEGF gene transfer
Serial levels of VEGF determined by ELISA disclosed a transient elevation 1 to 2 weeks after intramuscular (phVEGF\textsubscript{165}) gene transfer.
Induction of angiogenesis in ischemic heart

Delivery of:

- Proangiogenic proteins
- Genes coding for proangiogenic proteins
- Endothelial progenitor cells
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>
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<td>VEGF$_{165}$</td>
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<td>Rajagopalan et al. (2002)$^{17}$ and Rajagopalan et al. (2003)$^{18}$</td>
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<td>Adenovirus</td>
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<td>Plasmid</td>
<td>Intramuscular for PAD</td>
<td>Morishita et al. (2004)$^{20}$</td>
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</tbody>
</table>

CAD, coronary artery disease; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; PAD, peripheral arterial disease; TO, thromboangiitis obliterans; VEGF, vascular endothelial growth factor.
Vascularization of eye
Anatomy and histological organization of the retina

Ganglion cell axons
Radially orientated ganglion cell axons exit the eye through the optic nerve (arrow).

Angiogram of adult retina
Blood vessels also radiate from the optic nerve (white arrow) to the periphery.

Retinal astrocytes
Retinal astrocyte meshwork resembling that of blood vessels.

Gariano and Gardner, Nature 2005
In humans, a circular avascular zone of about 450 µm at the fovea improves central vision by reducing light scatter from blood vessels.

Gariano and Gardner, Nature 2005
Diabetic retinopathy
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

- Macugen
- Usual Care

50% Benefit

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

Reoviruses:

Ras protein

Growth factors eg. EGF

EGFR-I

Gefitinib
Erlotinib

Mutations present

EGFR-I sensitivity ↑↓

Cell proliferation

PI3K
RAF
MEK
MAPK
AKT

PKR

Antiviral response
**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

Before

After

Ovary carcinoma 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
a melanocyte

Melanoma

Nodular Melanoma
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

- **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

*Dudley et al. Science 2002*
1. Receptor consists of two proteins (α i β), encoded by separate genes – both were cloned from lymphocytes of the cured patient.

2. Genes were introduced to the retroviral vector.
Scheme of gene therapy with TcR antiMART-1 gene delivered by means of retroviral vector
today (72 months after therapy)...

...2 patients do not have any symptoms of melanoma

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
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.

Healthy cells: Inactive promoter leads to survival.

Cancer cells: Active promoter leads to cytolysis.
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.
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

Tumor cells

Virus without E1B

P53+

No viral replication

P53-

Viral replication

Cells survive

Cytolysis + viral spread
Prostate cancer cells cultured in vitro and infected with E1B-deficient adenoviruses

Effects of treatments of patients suffering from head and neck cancer with ONYX-015

Days after injections

Fractions of patients free of progression

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

1. Virus attaches to and infects tumor cell.
2. Foreign gene(s) of interest expressed.
3. No new viruses are produced.

Diagram shows the process of viral therapy in tumor cells.
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.

*Patient treated with Gendicine*
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 polimerase, 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.

This year application was rejected by EMEA.
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
Ex-vivo gene therapy
– treatment of familial hypercholesterolemia

Cloned gene LDL-R

Gene transfer

Patient cells LDL-R

Some cells now LDL-R+

Select cells

Amplify

LDL-R+ cells

Return genetically modified cells to patient
First case of gene therapy for treatment of familial hypercholesterolemia

First patient:

28 years old woman with high level of cholesterol in the blood (482 mg/dL) caused by mutation in LDL-R gene (normal cholesterol level: 160-210 mg/dL). After partial hepatectomia hepatocytes were cultured in vitro, infected with retroviral vector encoding for LDL-R cDNA and injected back to the patient.

It was found that:

- Method is safe
- There are hepatocytes containing the transgene in the patient liver
- Cholesterol level in the blood decreased to 360 mg/dL; lipoprotein profil was improved (for at least 18 months)

First clinical trial for treatment of familial hypercholesterolemia

First group of patients:

Five patients with high cholesterol level in the blood caused by mutation in LDL-R gene

It was found that:

- Method is safe
- There are hepatocytes with the transgene in the liver of patients
- Cholesterol level decreased by ~20% in three of five patients


The obtained results did not justify further trials
Severe combined immunodeficiency diseases

Common characteristic: occurrence of the block in T cell differentiation, always associated with a direct or indirect impairment of B cell immunity.

- 17 distinct SCID phenotypes have been identified to date
- Mutations in 10 genes have been found to cause SCID

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<th>Disease</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Cells affected</th>
<th>Reference</th>
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<td>X-linked SCID</td>
<td>IL-2RG</td>
<td>Xq13</td>
<td>T− B+ NK−</td>
<td>Puck et al. (1997)</td>
</tr>
<tr>
<td>JAK3 SCID</td>
<td>JAK3</td>
<td>19p12</td>
<td>T− B+ NK−</td>
<td>Candotti et al. (1997)</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>ADA</td>
<td>20q13.11</td>
<td>T− B− NK−</td>
<td>Hirschhorn (1995)</td>
</tr>
<tr>
<td>Omenn syndrome</td>
<td>RAG</td>
<td>11p13</td>
<td>T− B− NK+</td>
<td>Villa et al. (1998)</td>
</tr>
</tbody>
</table>

IL-2RG, interleukin-2 receptor gamma chain; JAK3, Janus kinase 3; ADA, adenosine deaminase; RAG, recombinase-activating gene; NK, natural killer; SCID, severe combined immune deficiency.

X-SCID (SCID-X1) – 1 : 150 000, ADA deficiency – 1 : 375 000
Cytokines receptors

David Vetter - Bubble Boy

David has spent 12 years in a foil-protected environment. Finally has received the bone marrow transplantation from his sister, but unfortunately died due to Epstein-Barr virus infection.
Gene therapy effective in treatment of X-SCID

Ex Vivo Transduced CD34+ Cells Expressing GammaC-R for X-SCID

Progenitor cells → CD34+ Selection → CD34+ transduction → Fibronectin coated flasks → Mouse Retroviral Vector → Growth factors (SCF, Flt-3, IL-3, PEG-MDF) → CD34+ expressing gamma-c receptor
Gene therapy is efficient in treatment of X-SCID

Hematopoietic progenitor cells devoid of correct gene of γc chain of cytokine receptor

Gene therapy

Hematopoietic progenitor cells with a correct gene of γc chain of cytokine receptor

Retroviral vector with a correct gene of γc chain of cytokine receptor
Results of X-SCID gene therapy

SCID-X1:

1. French trial – 10 treated, 9 benefited. Unfortunately, four of those developed leukemia and one boy died because of leukemia.

2. British trial – 7 treated, 7 benefited – one developed leukemia.

Development of uncontrolled clonal T lymphoproliferative syndrome, similar to acute lymphoblastic leukemia in 3 children due to the integration of a vector into an LMO2 gene either close to the promoter or in the first intron. Potential reasons:

- LMO-2 locus is a frequent site for retroviral integration (not supported by the data)

- Cells with aberrant expression of LMO-2 could have been selected because they provide a clonal growth advantage
First controlled trial of gene therapy - 1990

ADA deficiency – results in severe immunodeficiency syndrome
ADA deficiency

Deoxy-adenosine
adenosine

Deoxy-inosine
inosine

Due to inhibition of T cell differentiation in the thymus
Gene therapy of ADA deficiency

Cloned gene

ADA

Cells removed

Gene transfer

Patient cells ADA⁻

Some cells now ADA⁺

Select cells

Amplify

Return genetically modified cells to patient

ADA⁺ cells
First clinical trial of gene therapy - 1990

Ashanti De Silva (patient)

Retroviral vector containing correct ADA gene (cDNA) has been transduced into blood lymphocytes

This first clinical trial was not „pure” from the methodological point of view.

The patients have been treated concomitantly with enzyme injections – ADA-PEG.

Nevertheless, the marker transgene (neo) could be detected in the blood cells of the patients even more than 12 years after injection of modified cells.
Gene therapy of ADA deficiency

Five gene therapy clinical trials have been performed since 1990

1. First two trials: repeated infusions of peripheral T cells, ex vivo transduced with ADA-encoding retroviral vector. Additionally, enzymatic substitution of PEG-ADA has been performed in those patients
   - first two patients: transduced cells have been detected 12 years after procedure
   - another trial: six patients: in one patient PEG-ADA was discontinued, what led to a selective growth advantage of ADA-gene transduced T lymphocytes

2. Other three trials – transduction of HSC - but with reduction or even abrogation of PEG-ADA

In the 14 patients treated so far, the correction of T and B cell immunodeficiency has enabled a normal life up to three years after treatment, and not serious adverse effects have been reported.
Thank you :}

[Image of cells or molecules]