Angiogenesis in diseases
Anti – and pro-angiogenic therapies

lecture V
30th March 2015
Physiological angiogenesis in adults is restricted

- Placenta
- Uterus
- Hair growth
- Wound healing
Tumor growth is dependent on angiogenesis.
Elongation of capillary by 1 mm allows for growth of $\sim 10,000$ cancer cells
Tumor Angiogenesis: A Balancing Act

Judah Folkman, 1933-2008

Endogenous angiogenesis inhibitors

Angiogenesis promoters

Angiogenesis is dependent on the balance between pro- and anti-angiogenic mediators.
The Angiogenic Switch is necessary... for Tumor Growth and Metastasis

- **Tumor is dormant**
- **Angiogenic switch**

Somatic mutation → Small avascular tumor → Tumor secretion of angiogenic factors stimulates angiogenesis → Rapid tumor growth and metastasis

Neovascularization:
- Allows rapid tumor growth by providing oxygen, nutrients, and waste removal
- Facilitates metastasis

The balance hypothesis for the angiogenic switch

Hanahan & Folkman, 1996
• Removes waste from growing tumor

• Provides tumor cells with oxygen and nutrients to grow
Tumor Cells

Endothelial Cells

Extracellular Matrix

Basement Membrane

VEGF

bFGF

Supporting cells

Extracellular Matrix

proteases

PDGFR

PDGF

Supporting cells

Basement Membrane

Endothelial Cells
Vasculogenic mimicry

- Vasculogenic mimicry (VM) was introduced in 1999 and described the unique ability of highly aggressive melanoma cells to dedifferentiate into multiple cellular phenotypes, and obtain endothelial-like characteristics.

- This process would then lead to the formation of de novo vasculogenic-like matrix-embedded networks, i.e. **vascular-like structures**, containing plasma and red blood cells, and ultimately contributing to blood circulation.
Blood vessels in tumors are different than in healthy tissue: view from outside.
Blood vessels in tumors are different than in healthy tissue: view from outside

Vessels of healthy tissue
Vessels of tumor
Blood vessels in tumors are different than in healthy tissue.
Tumor vessels are leaky
The major differences between normal and tumor vasculature

**NORMAL VASCULATURE**
- well organized structure
- defined hierarchy: arteriole-capillary-venule
- pericytes in close contact with endothelial cells
- basement membrane present
- normal blood flow
- non-permeable vessels
- low interstitial pressure

**TUMOR VASCULATURE**
- disorganized structure
- lack of arteriole-capillary-venule hierarchy
- lack of pericytes
- absence of basement membrane
- impaired blood flow
- highly permeable vessels
- high interstitial pressure
• antiangiogenic therapy is a relatively new form of treatment using drugs called 'angiogenesis inhibitors' that specifically halt new blood vessel growth

• the idea of the therapy: tumors are starved by cutting off its blood supply
The influence of angiogenesis inhibition on tumor growth

Tumor without treatment

Tumor increases and metastases

Tumor treated with antiangiogenic therapy

Tumor decreases
Antiangiogenic Therapy

Hundreds of angiogenesis inhibitor molecules have been discovered so far:

• Some angiogenesis inhibitors are naturally present in the human body, because healthy tissues appear to resist cancer growth by containing these antiangiogenic compounds.

• Other angiogenesis inhibitors have been found in nature - in green tea, soy beans, fungi, mushrooms, tree bark, shark tissues, snake venom and many other substances.

• Still other angiogenesis inhibitors have been manufactured synthetically in the laboratory.

• Some FDA-approved medicines have also been "re-discovered" to have antiangiogenic properties.

Currently, at least 40 antiangiogenic drugs are being tested in clinical trials.
Proteins or fragments of proteins that are formed in the body, which subsequently can inhibit the formation of blood vessels by disrupting the angiogenic process

They are
• present in the circulation
• sequestered in the ECM surrounding cells
# List of Angiogenesis Inhibitors in the Body

<table>
<thead>
<tr>
<th>Angiostatin (plasminogen fragment)</th>
<th>Metalloproteinase inhibitors (TIMPs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-angiogenic antithrombin III (aaATIII)</td>
<td>Pigment epithelial-derived factor (PEDF)</td>
</tr>
<tr>
<td>Canstatin</td>
<td>Placental ribonuclease inhibitor</td>
</tr>
<tr>
<td>Cartilage-derived inhibitor (CDI)</td>
<td>Plasminogen activator inhibitor</td>
</tr>
<tr>
<td>Endostatin (collagen XVIII fragment)</td>
<td>Prolactin 16kD fragment</td>
</tr>
<tr>
<td>Fibronectin fragment</td>
<td>Proliferin-related protein</td>
</tr>
<tr>
<td>Heparinases</td>
<td>Retinoids</td>
</tr>
<tr>
<td>Heparin hexasaccharide fragment</td>
<td>Thrombospondin-1</td>
</tr>
<tr>
<td>Human chorionic gonadotropin (hCG)</td>
<td>Transforming growth factor-beta</td>
</tr>
<tr>
<td>Interferon alpha/beta/gamma</td>
<td>Tumistatin</td>
</tr>
<tr>
<td>Interferon inducible protein (IP-10)</td>
<td>Vasculostatin</td>
</tr>
<tr>
<td>Interleukin-12 (IL-12)</td>
<td>Vasostatin (calreticulin fragment)</td>
</tr>
<tr>
<td>Kringle 5 (plasminogen fragment)</td>
<td>Angioarrestin</td>
</tr>
</tbody>
</table>
What is the mechanisms of actions of anti-angiogenic drugs?
Mechanism of action of anti-angiogenic agents

Antiangiogenic drugs will stop the formation of blood vessels and blood flow in tumors.
Normalisation of blood vessels as the mechanisms of action of anti-angiogenic agents

normal  tumor  anti-angiogenic therapy

Jain, Science 2004
Different stages of angiogenesis inhibition

- Inhibition of VEGF activity
- Inhibition of late stages of angiogenesis (angiostatin, endostatin)
- Inhibition of matrix degradation and endothelial cells migration
- Antibody neutralization
- Soluble VEGFR
- VEGFR tyrosine kinase inhibitors
- anti-α₅β₃
- anti-MMPs
- Metastasis

Chemistry & Biology
Various strategies to inhibit VEGF signaling

a) Anti-VEGF antibodies
b) Anti-VEGF-1 antibodies
c) Anti-VEGFR-2 antibodies
d) Soluble VEGF receptors
e) Small-molecule VEGFR TK inhibitors
f) Aptamers

Ferrara and Kerbel, Nature 2005
Endostatin

• It was first discovered in 1995 in Dr. Folkman’s lab

• Phase I clinical studies began in 1999

• A naturally-occurring 20-kDa C-terminal fragment derived from type XVIII collagen.

• Interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF)
Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth

Michael S. O'Reilly,* Thomas Boehm,* Yuen Shing,* Naomi Fukai,† George Vasios,§ William S. Lane,‡ Evelyn Flynn,* James R. Birkhead,§ Bjorn R. Olsen,† and Judah Folkman*

*Department of Surgery
Endostatin treated Lewis Lung Carcinoma

A) Proliferation

PCNA (%)

Saline-treated | Endostatin-treated

B) Apoptosis

TUNEL (%)

Saline-treated | Endostatin-treated

C) Angiogenesis

Vessels / hpf

Saline-treated | Endostatin-treated
Endostar, a recombinant human endostatin, has a broad spectrum of activity against solid tumors. In this study, we aimed to determine whether the anticancer effect of Endostar is increased by using a nanocarrier system. It is expected that the prolonged circulation of endostar will improve its anticancer activity. To study the effects of endostar-loaded nanoparticles in vivo, nude mice in which tumor cells HT-29 were implanted, were subsequently treated with endostar or endostar-loaded PEG-PLGA nanoparticles.

A) Endostar-loaded PEG-PLGA nanoparticle group.
B) Endostar group.
C) Blank PEG-PLGA nanoparticle group.
D) Phosphate-buffered saline blank control group.

Simgere Pharmaceutical Group Successfully Completes Endostar Phase IV Clinical Study

Combining Endostar with standard chemotherapy regimes can significantly improve the survival of patients with advanced NSCLC with no significant increase in adverse effects from chemotherapy.
How Avastin Starves a Tumor

Tumors need blood, and they have a devious way to get it:

- They secrete a protein called VEGF that docks with receptors in nearby blood vessels, stimulating the growth of new blood vessels.

- Genentech foils this plot with Avastin, a drug that binds with VEGF and prevents that protein from attaching to receptors. New blood vessels don't form, and the tumor starves.
Angiogenesis inhibitors in clinical trials

10 000 publications in Pubmed
Avastin

• Avastin is a humanized monoclonal antibody (MAb) that targets vascular endothelial growth factor (VEGF)

• Causes regression of tumor vasculature

• Reduces intra-tumor pressure, thereby improving the delivery of cytotoxic agents to the tumor

• Also inhibits new tumor blood vessel formation, restricting tumor growth

• The first anti-angiogenic agent with demonstrated anticancer benefit in phase III trials
Anti-VEGF antibody Bevacizumab (Avastin)
Inhibition of experimental human tumor growth by anti-VEGF antibody (precursor of Avastin)

VEGF Ab  control Ab

Experimental hepatic metastases
Avastin in clinical development: overview

**Phase I**
- Combined with 5-FU/LV in previously untreated mCRC
  - 5 or 10mg/kg every 2 weeks (n=25)
  - Kabbinavar F, et al. 2003
- Combined with CP in previously untreated NSCLC
  - 3mg/kg every week (n=12)
  - Margolin K, et al. 2001
- Combined with chemotherapy in metastatic cancers
  - 3mg/kg every week (n=12)
  - Margolin K, et al. 2001

**Phase II**
- Combined with IFL in previously untreated mCRC
  - 5mg/kg every 2 weeks (n=813)
  - Hurwitz H, et al. 2004
- Combined with 5-FU/LV in previously untreated mCRC
  - 5mg/kg every 2 weeks (n=209)
  - Kabbinavar F, et al. 2005
- Combined with CP in previously untreated NSCLC
  - 3 or 10mg/kg every 2 weeks (n=116)
  - Yang JC, et al. 2003
- Combined with gemcitabine in unresectable PC
  - 10mg/kg every 2 weeks
  - Kindler HL, et al. 2004

**Phase III**
- Combined with either FOLFOX4 or XELOX in previously untreated mCRC (n=1,920)
- Combined with either FOLFOX4 or XELOX in previously untreated mCRC
  - 10mg/kg every 2 weeks (n=638)
- Combined with Xeloda® in previously treated MBC
  - 15mg/kg every 3 weeks (n=462)
  - Miller KD, et al. 2005
- Combined with Tarceva™ + gemcitabine in previously untreated PC
  - 5mg/kg every 2 weeks (n=600)
A Randomized Trial of Bevacizumab, an Anti-Vascular Endothelial Growth Factor Antibody, for Metastatic Renal Cancer

James C. Yang, M.D., Leah Haworth, B.S.N., Richard M. Sherry, M.D., Patrick Hwu, M.D., Douglas J. Schwartzentruber, M.D., Suzanne L. Topalian, M.D., Seth M. Steinberg, Ph.D., Helen X. Chen, M.D., and Steven A. Rosenberg, M.D., Ph.D.
Increase in survival of patients with renal cell cancer treated with Avastin

Progression free survival (%)

- High dose
- Low dose
- Placebo

Months from on-study date

Fever, hypertension, proteinuria – adverse effects

Two-sided unadjusted p values:
- High dose versus low-dose: p=0.0821
- Low dose versus placebo: p= 0.0115

High dose versus placebo: p<0.001
Avastin in clinics

• The FDA approved Avastin in February 2004 for use in combination with intravenous 5-Fluorouracil (5-FU)-based chemotherapy as a treatment for patients with first-line metastatic cancer of the colon or rectum.

• In June 2006, the FDA approved Avastin for use in combination with intravenous 5-FU-based chemotherapy for patients with second-line metastatic cancer of the colon or rectum.
Avastin in clinics

• In October 2006, the FDA approved Avastin in combination with carboplatin and paclitaxel for the first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous, non-small cell lung cancer.

• Nowadays, it is also approved for treatment of metastatic renal cancer in combination with interferon alpha.

• Glioblastoma that has progressed after prior treatment can be also treated with Avastin.
Are inhibitors of VEGF signaling used only in cancer treatment?

Angiogenesis inhibitors are being used to treat some diseases that involve the development of abnormal blood vessel growth in non-cancer conditions, such as macular degeneration.
What is AMD?

- Age-related macular degeneration (AMD) is defined as the loss of macular function from the degenerative changes of aging.
- The macula is the most important part of the retina responsible for sharp, central vision.
- AMD is divided specifically into two distinct types: the less severe or “dry” form, and the more severe and debilitating “wet” form.

Drusen (tiny yellow or white accumulations of extracellular material) formation under the Macula.

Macula with abnormal blood vessels.
Age-related macular degeneration (AMD)
Current Treatments for AMD...

- **Lucentis™ (ranibizumab)** — The FDA approved Lucentis in June 2006 for the treatment of wet AMD.

  - Lucentis (ranibizumab) is a humanized anti-VEGF antibody fragment (a fragment of Avastin) that inhibits VEGF activity by competitively binding with VEGF.

  - A two-year study showed that **95 percent** of people with wet AMD who received monthly injections of Lucentis experienced no significant loss in visual acuity.
Avastin is a cancer drug marketed by Roche (Genentech), but some pharmacies repackage Avastin into smaller units to treat an eye condition like macular degeneration. Roche has long warned against the unapproved use of Avastin in the eyes, and the company markets a similar drug called Lucentis that was specifically designed and approved for use in the eyes. However, some eye doctors use Avastin because a much smaller dose is needed compared with that required for a cancer patient. An injection of repacked Avastin costs about $50, while an injection of Lucentis tops $1,000.

20 Mar 2013 - FDA Warns of Eye Infections From Unapproved Avastin (The Wall Street Journal)

It is not the first time repacked Avastin syringes have been associated with eye infections. In 2011, the FDA warned about a cluster of serious eye infections in Florida linked to Avastin syringes made by a pharmacy in that state.
In France....

**France aims to save big bucks by subbing unapproved Avastin for eye drug Lucentis**

July 2, 2014 | By Arlene Weintraub

In June, the Italian government said it would pay for Roche's ($RHBBY) cancer drug Avastin to be used to treat a blinding eye disease, in place of the company's far more expensive eye drug Lucentis. Now, France is following Italy's lead.

The French legislature has introduced an amendment to its social security budget bill that would green-light the use of Avastin in age-related macular degeneration (AMD), even though the drug is not approved for that use. Lawmakers will vote on the measure next week. The move would affect both Roche and its Lucentis marketing partner in Europe, Novartis ($NVS).

And it's backed up by some compelling economic arguments. Gerard Bapt, one of France's socialist legislators, estimates that Avastin is 30 times less expensive than Lucentis in that country. A positive vote on the measure could save France at least €200 million ($273 million) a year, Bapt estimates, according to Reuters.
Pegaptanib – Macugen

Aptamer which binds VEGF-165

**VEGF** binds to its receptors on the cell surface and stimulates angiogenesis.

**Macugen** binds to VEGF. This prevents VEGF from binding to the receptors on the cell surface. As a result, angiogenesis is not stimulated.
V.I.S.I.O.N. trial results

Early and Sustained Treatment Benefit

- Macugen
- Usual Care

50% Benefit
*P<0.01

Weeks:
0  6  12  18  24  30  36  42  48  54
Macugen was demonstrated to be effective in prevention of vision loss in two large clinical trials in patients with AMD.
Macugen (aptamer blocking VEGF) – antiangiogenic drug approved for treatment of ophthalmological diseases

Inhibition of VEGF binding to VEGF receptors in cells treated with macugen
Aflibercept (AVE0005)

- Composite decoy receptor based on VEGFR-1 and VEGFR-2 fused to a human Fc segment of IgG1 that binds VEGF
- Decreases free VEGF to bind to receptors and prevent vessel growth
- FDA approved for macular degeneration
Afibercept – VEGF-Trap

VEGF Trap (Regeneron Pharmaceuticals) is a high-affinity recombinant fusion protein that consists of the immunoglobulin domain 2 of the VEGF-R1 receptor and the domain 3 of the VEGF-R2 receptor, fused to the crystallizable fragment of human IgG.

It has a high affinity for VEGF-A and PIGF; it is more tightly bound to VEGF-A and PIGF than native receptors.
VEGF-trap

Twice Weekly Dosing with VEGF-Trap Effectively Inhibits Subcutaneous Growth of a Variety of Tumor Types

Control 25mg/kg | Control 25mg/kg | Control 25mg/kg 2.5mg/kg

Mouse B16F10.9 Melanoma | Human A673 Rhabdomyosarcoma | Rat C6 Glioma

Hallosh et al. 2002
VEGF Trap-Eye is a fully human, soluble recombinant decoy VEGF receptor that is biologically engineered to bind to all forms of this growth factor or including PlGF and block it from binding to the target receptors.

Blocking VEGF can prevent abnormal blood vessel formation as well as vascular leak and has proved beneficial in the treatment of wet AMD.

VEGF Trap-Eye contains ultrapurified aflibercept (VEGF Trap) and has been specifically developed as an iso-osmotic solution for injection into the eye.

Unlike currently available anti-VEGF agents, VEGF Trap-Eye inhibits placental growth factor 1 (PlGF1) in addition to all isoforms of VEGF-A. PlGF is another member of the VEGF family also believed to be implicated in the development of wet AMD.

Because the binding affinity of VEGF Trap-Eye for VEGF-A isoforms and PlGF1 is higher than that of native receptors VEGFR1 and VEGFR2, it effectively blocks VEGF binding and activation of native receptors.
Ramucirumab (IMC-1121B, trade name Cyramza) is a fully human monoclonal antibody (IgG1) developed for the treatment of solid tumors. It is directed against the VEGFR2.

On April 21, 2014, the FDA approved Ramucirumab as a single-agent for the treatment of patients with advanced or metastatic, gastric or gastro-esophageal junction adenocarcinoma with disease progression on or after prior fluoropyrimidine- or platinum-containing chemotherapy.

On September 26, 2013, the manufacturer Eli Lilly announced that its Phase III study for ramucirumab failed to hit its primary endpoint on progression-free survival among women with metastatic breast cancer.

In June 2014, the drug failed its second phase III trial - no statistical significance in the overall survival of patients with liver cancer.
Other angiogenesis inhibitors
## Table 2 – Anti-VEGF therapeutic agents currently in development for breast cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism(s) of action</th>
<th>Molecular target(s)</th>
<th>Stage of clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>Anti-VEGF antibody</td>
<td>VEGF ligand</td>
<td>Approved</td>
</tr>
<tr>
<td>Sorafenib (BAY 43-9006)</td>
<td>Tyrosine kinase inhibitor</td>
<td>Raf-1, VEGF receptors-2 and -3, PDGFR-β, Flt-3, c-Kit</td>
<td>Phase II</td>
</tr>
<tr>
<td>Sunitinib (SU11248)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, Flt-3, PDGFR-α, PDGFR-β, c-Kit</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>Vatalanib (PTK/ZK)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, PDGFR-β, c-Kit, c-Fms</td>
<td>Phase II</td>
</tr>
<tr>
<td>Vandetanib (ZD6474)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-2 and -3, EGFR</td>
<td>Phase II</td>
</tr>
<tr>
<td>Motesanib (AMG-706)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, PDGFR, c-Kit</td>
<td>Phase II</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1 and -2, PDGFR-β, and c-Kit</td>
<td>Phase II</td>
</tr>
<tr>
<td>Axitinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, PDGFR, c-kit</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

VEGF, vascular endothelial growth factor; PDGFR, platelet-derived growth factor receptor.

Marty et al. 2008
Anti-angiogenic drugs - summary

Cancer epithelia - Cancer tissue

Autocrine/paracrine

Cancer tissue

Autocrine/paracrine

VEGF

VEGFR-2

TKIs

Bevacizumab

Ramucirumab

VEGF
Other diseases...

psoriasis
Angiogenesis drives psoriasis pathogenesis

- hyperplastic and inflammed dermal blood vessels
- epidermal thickening (acanthosia) with aberrant keratinocyte proliferation
- inflammatory infiltrates
Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis

Yu-Ping Xia, Baosheng Li, Donna Hylton, Michael Detmar, George D. Yancopoulos, and John S. Rudge

Figure 2. Psoriasiform phenotype. Erythematous, scaly, and thickened skin lesions with associated edema develop in homozygote K14-VEGF transgenic mice older than 5 months.
Simultaneous deletion of JunB and c-Jun (DKO*) in the epidermis of adult mice leads to a psoriasis-like phenotype with hyper- and parakeratosis and increased subepidermal vascularization.

For treatment mice received anti-VEGF antibodies to inhibit VEGF signaling. These results demonstrate that systemic blockade of VEGF by an inhibitory antibody might be used to treat patients who have inflammatory skin disorders such as psoriasis.
Table 2 Modern biologics and small molecules targeting angiogenesis directly or by indirect pathways

<table>
<thead>
<tr>
<th>Anti-psoriatic therapeutics interacting with EC biology</th>
<th>Anti-angiogenic mechanism</th>
<th>Clinical relevance for psoriasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efalizumab</td>
<td>Inhibits the transmigration of T cells by blocking the binding of LFA-1 to ICAM-1</td>
<td>Approved</td>
</tr>
<tr>
<td>Etanercept</td>
<td>TNF-antagonist, reduces VEGF levels</td>
<td>Approved</td>
</tr>
<tr>
<td>Infliximab</td>
<td>TNF-antagonist, reduces VEGF, angiopoietin and Tie-2 expression</td>
<td>Approved</td>
</tr>
<tr>
<td>Fumaric acid esters</td>
<td>Inhibit TNF-mediated nuclear entry of NF-κB p65 in ECs</td>
<td>Approved (in Germany)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Therapeutics directly targeting angiogenesis</th>
<th>Anti-angiogenic mechanism</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IL-8</td>
<td>Inhibits capillary tube formation <em>in vitro</em></td>
<td>No efficacy in phase IIb study</td>
</tr>
<tr>
<td>Neovastat</td>
<td>Inhibits EC proliferation and the activity of specific MMPs</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Kinase inhibitor targeting VEGFR, PDGFR and FGFR</td>
<td>Case report</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Kinase inhibitor targeting VEGFR, PDGFR and KIT</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

LFA-1, lymphocyte function-associated antigen-1; MMPs, matrix metalloproteinases; TNF, tumour necrosis factor; ECs, endothelial cells; VEGF, vascular endothelial growth factor; PDGFR, platelet-derived growth factor receptor; VEGFR, VEGF receptors; FGFR, fibroblast growth factor receptor.
Treatment of numerous diseases can be improved by pro-angiogenic therapy... 

Treatment of numerous diseases can be improved by anti-angiogenic therapy
Pro-angiogenic therapy

• one of the possible therapy for cardiovascular disorders

• one of the first trial: fibroblast growth factor - bFGF was used to treat patients with coronary heart disease (Stegmann et al. 2000)

…..The first clinical study on patients with coronary heart disease treated by local intramyocardial injection of FGF-1 showed a 3-fold increase of capillary density mediated by the growth factor. Also, angiogenic growth factor injection intramyocardially as sole therapy for end-stage coronary disease showed an improvement of myocardial perfusion in the target areas as well as a reduction of symptoms and an increase in working capacity. Angiogenic therapy of the human myocardium introduces a new modality of treatment for coronary heart disease in terms of regulation of blood vessel growth. Beyond drug therapy, angioplasty and bypass surgery, this new approach may evolve into a fourth principle of treatment of atherosclerotic cardiovascular disease…..
Pro-angiogenic therapy

Delivery of the angiogenic agents: protein, gene and cell therapies.

1. In protein therapy, recombinant proteins are used directly to induce therapeutic effects. However, a major limitation of this approach is the very short half-life of exogenous proteins in target tissues, resulting in only transient therapeutic effects.

2. In contrast, gene therapy uses non-viral or viral vectors to carry a gene construct encoding a therapeutic protein into target tissues, where it is abundantly expressed by the target cells.

3. The idea of cell therapy in its present form is that transplanted cells function as protein factories with the capability of producing multiple endogenous growth factors, meaning that the transplanted cells will induce vascular growth mainly in a paracrine manner, rather than directly replacing damaged cells.
Gene therapy for cardiovascular disorders

Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 64.1% (n=1331)
- Monogenic diseases 9.1% (n=188)
- Infectious diseases 8.2% (n=170)
- Cardiovascular diseases 7.8% (n=162)
- Neurological diseases 1.8% (n=37)
- Ocular diseases 1.6% (n=33)
- Inflammatory diseases 0.7% (n=14)
- Other diseases 1.8% (n=38)
- Gene marking 2.4% (n=50)
- Healthy volunteers 2.6% (n=53)

The Journal of Gene Medicine, © 2014 John Wiley and Sons Ltd

www.wiley.co.uk/genmed/clinical
Pro-angiogenic therapy

- Therapeutic angiogenesis with VEGF is a promising approach for the treatment of ischemic myocardium and peripheral skeletal muscles.

- Preclinical studies in large animals have clearly demonstrated safety and efficacy of VEGF gene therapy. However, first clinical trials with intravascular delivery of VEGF vector constructs have only resulted in limited benefits to the patients.

- In order to achieve better transfection efficiency and more targeted effects trials based on direct intramyocardial and intraskeletal muscle injections are performed.

- Phase I/II studies are currently ongoing to test safety, feasibility and efficacy of these improved approaches in patients with severe cardiovascular diseases.
VEGF therapy for ischemia-related diseases

- Femoral artery ligation

- VEGF plasmid injection

- Rabbits, 2 weeks

- Plasma VEGF
- Local VEGF mRNA and protein
- Blood flow in muscle
- Number of microvessels
Effect of gene $\text{VEGF}_{165}$ gene transfer on the capillary density in the rabbit adductor muscle

Control leg

Ischemic leg injected with $\beta$-galactosidase

Ischemic leg injected with pVEGF165

(14 days after injection)
VEGF gene transfer increases blood flow.

![Bar chart showing mean local blood flow in adductor muscle (100% - blood flow before ischemia) for β-gal and VEGF. The chart indicates that VEGF significantly increases blood flow compared to β-gal with a p-value less than 0.02.]

![Images showing blood flow in ischemic leg: Immediately after injection and 14 days after injection, comparing VEGF and β-gal.]

*References:*
- Dulak et al., Eur Surgery 2002, 34: 105-110
FGF4 and VEGF transfer restores blood flow in ischemic leg

Jazwa et al., Vasc Cell 2013
VEGF-A gene transfer stimulates neovascularization in mouse muscles

Representative pictures of FITC-lectin-stained (green) capillaries 21 days after AAV-mediated gene transfer.
Physiological angiogenesis in adults is restricted, however disturbances of this process is a hallmark of many diseases.

One of the strategy to treat tumor/AMD is the anti-angiogenic therapy.

Angiogenesis inhibitors specifically halt new blood vessel growth and starve a tumor by cutting off its blood supply.

Angiogenesis inhibitors prevent the VEGF from binding with the receptors on the surface of the endothelial cells but they may also act on VEGFR or TK activity.

Many anti-angiogenic factors are in the Phase II/III of clinical trails.

On the other hand, the proangiogenic therapy is a possible way of treatment of cardiovascular disorders.