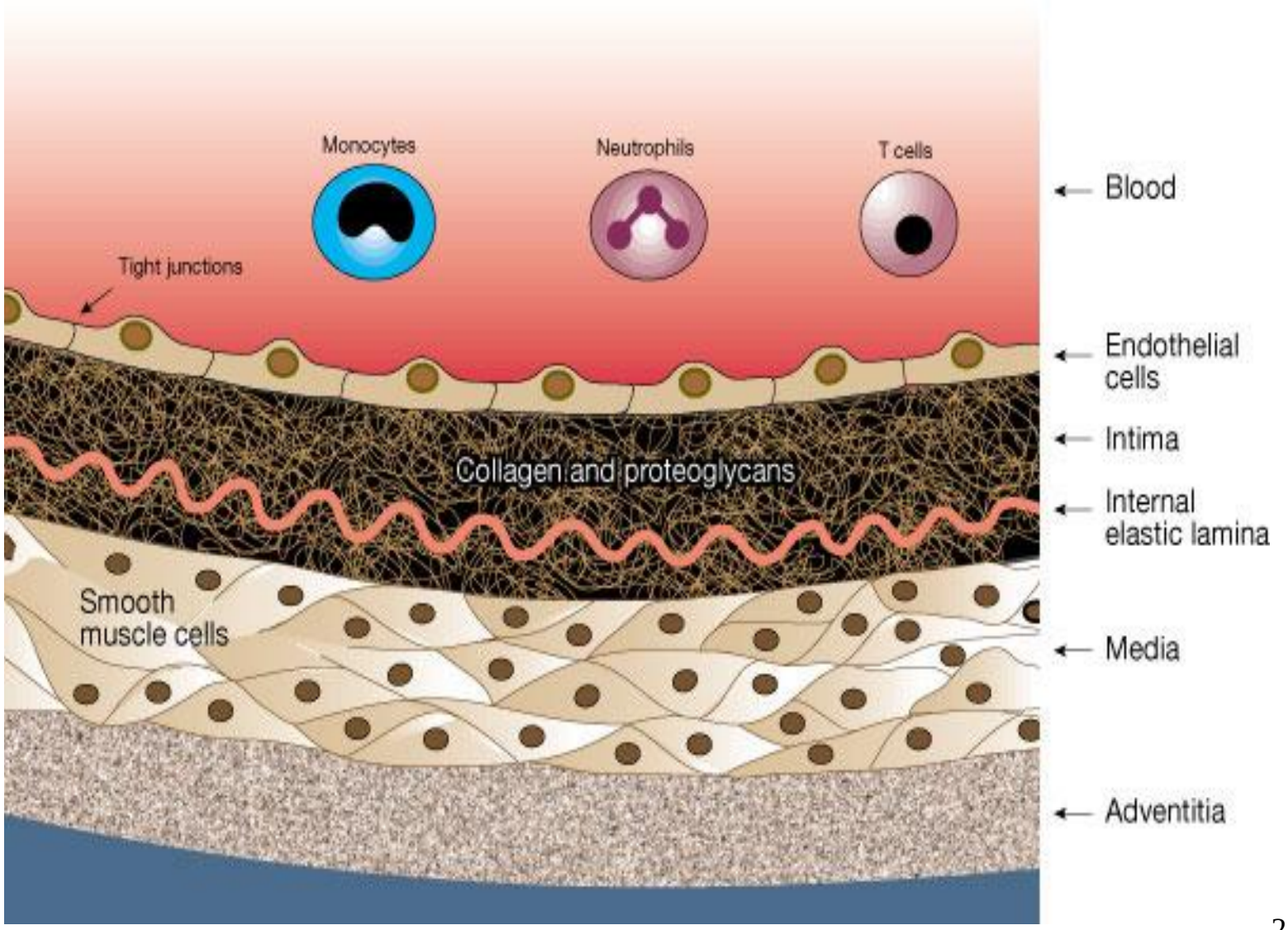
Lecture 11

Gene therapy of cardiovascular diseases

20.12.2011

Structure of a normal large artery



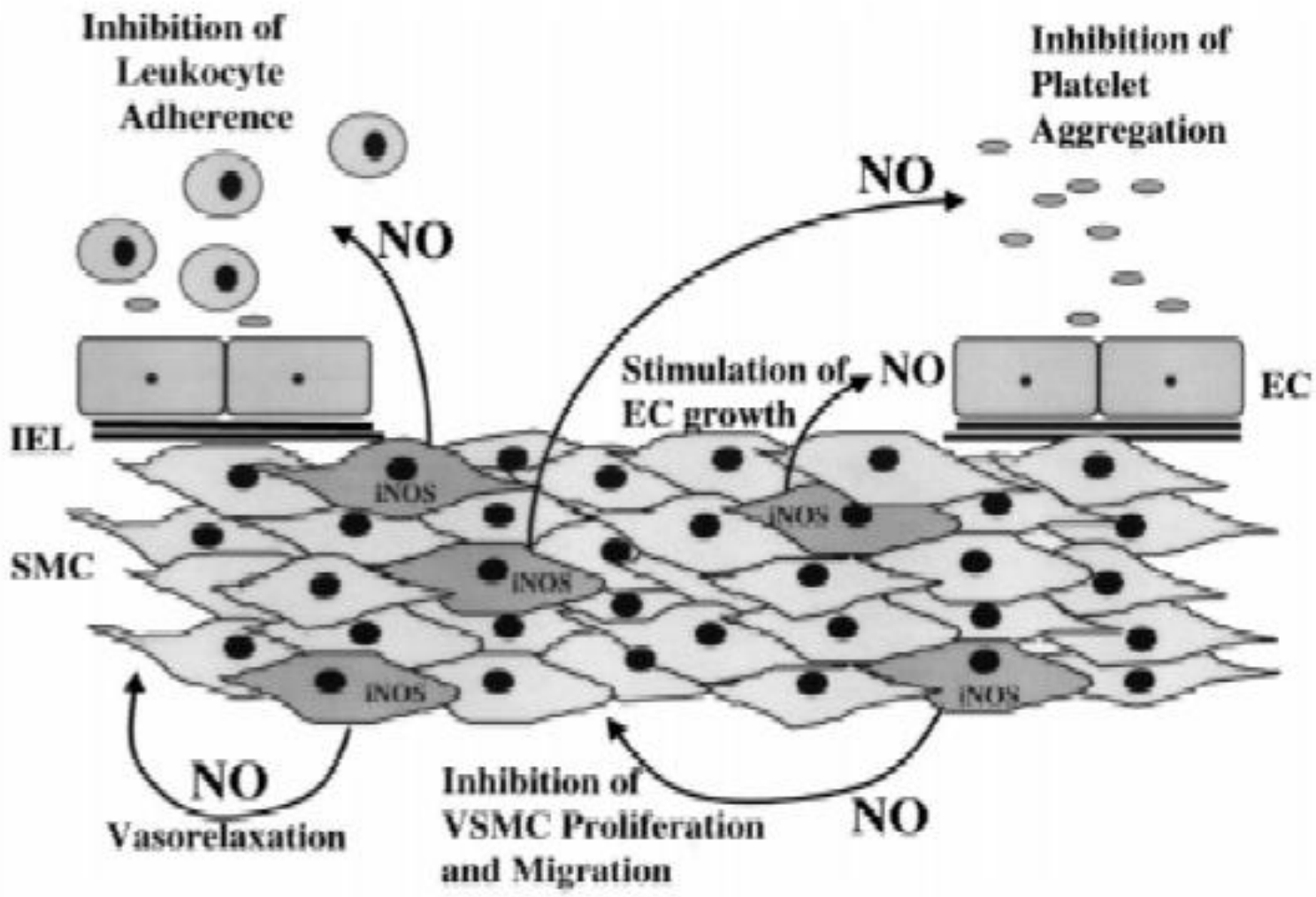
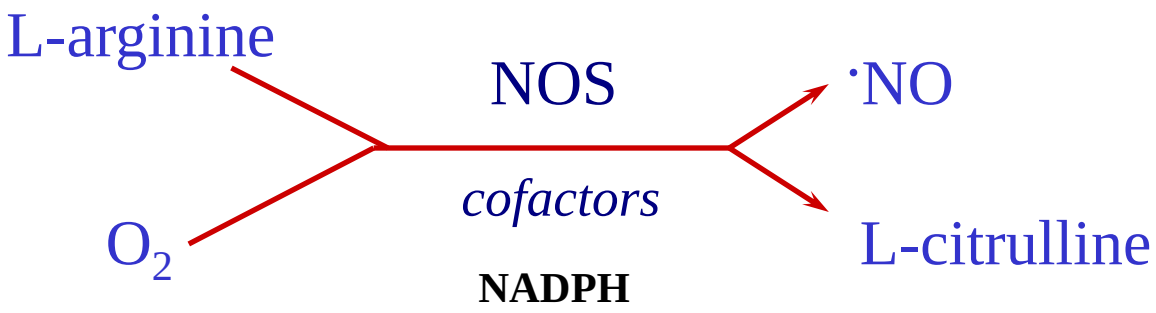
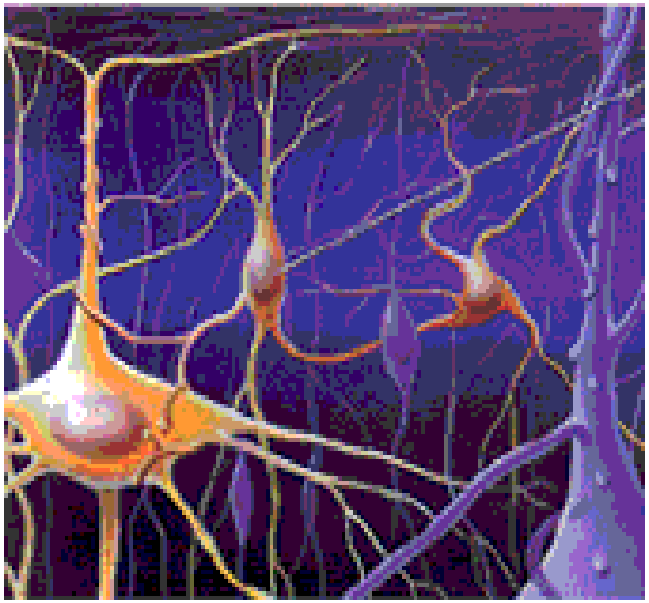


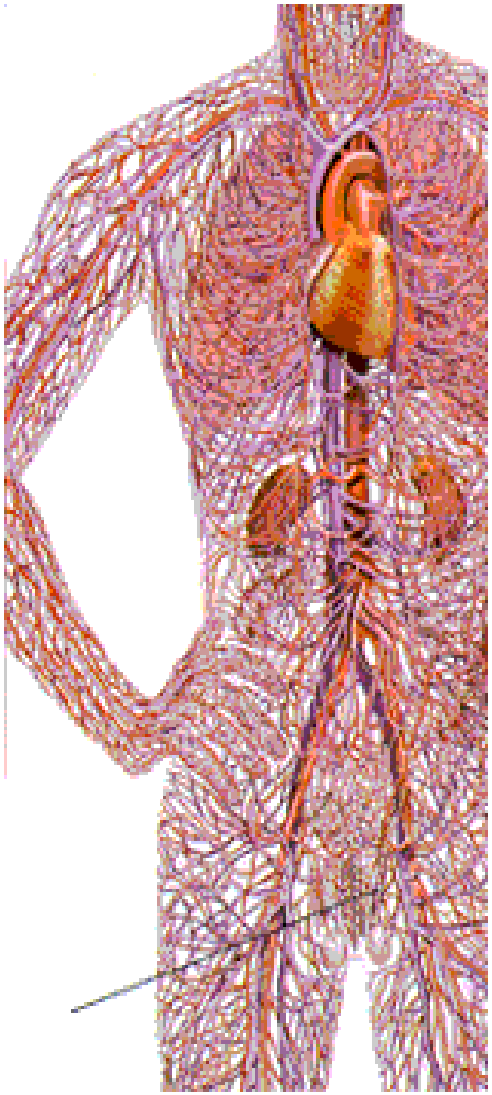
Fig. 1. The vasoprotective effects of nitric oxide. Nitric oxide (NO) production at the site of injury can inhibit platelet aggregation and adherence, leukocyte adherence, vascular smooth muscle cell (VSMC) proliferation, and VSMC migration. Additionally, NO can stimulate endothelial cell proliferation and protect the endothelial cells from apoptosis. These properties of NO favor reestablishment of a normal vascular environment following arterial injury.


L-arginine
NOS
·NO
cofactors
O2
L-citrulline
NADPH

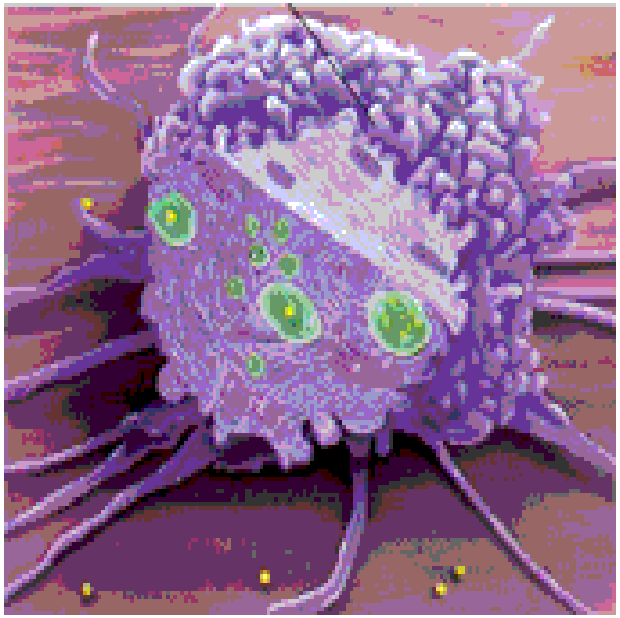
Nitric oxide is ubiquitous



Nervous system



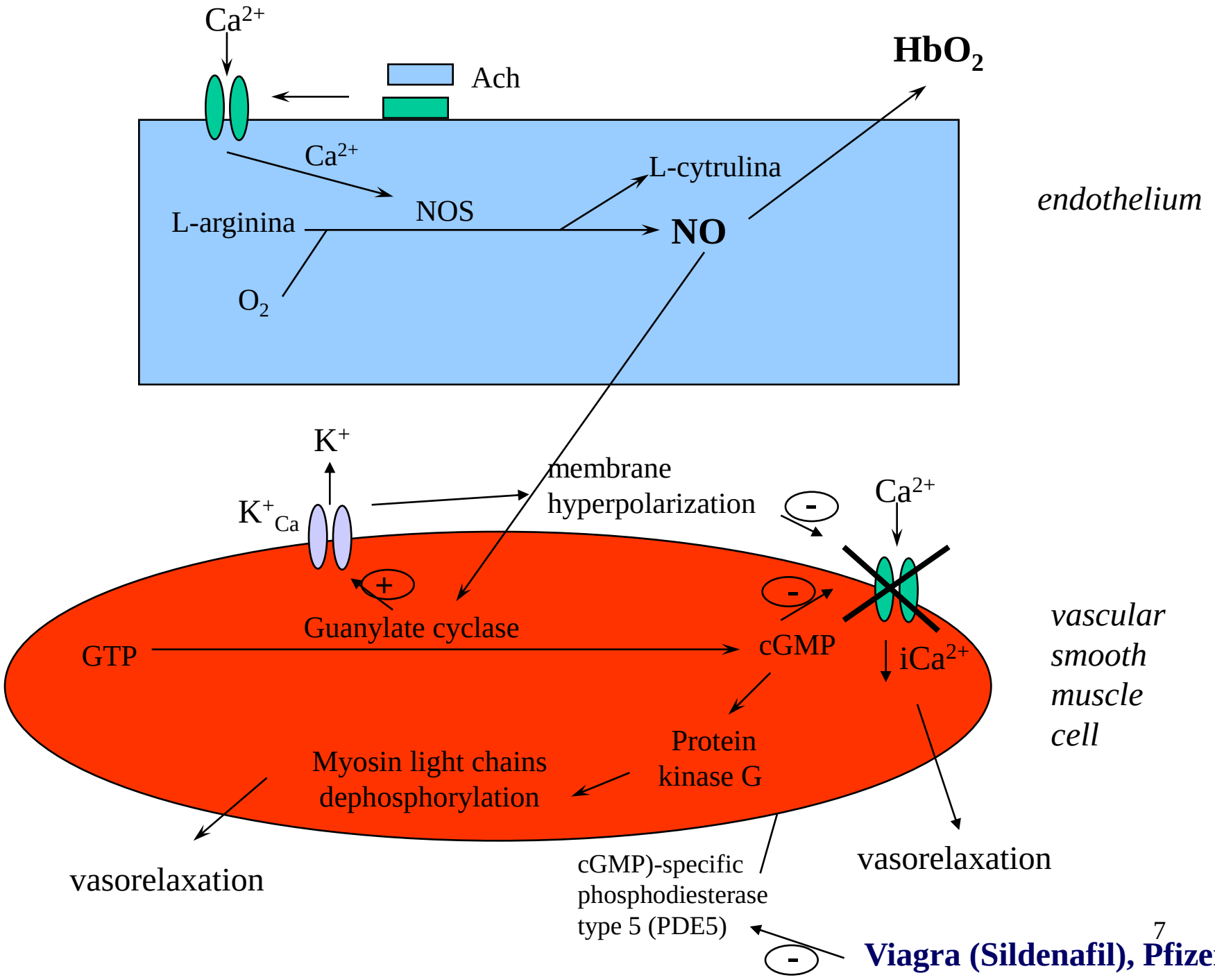
cardiovascular system



defense system

Nitric oxide synthases

eNOS - endothelial (constitutive) NOS (NOS III)
nNOS - neuronal (constitutive) NOS (NOS I)
iNOS - inducible (NOS II)


Ca2+
Ach
HbO2
Ca2+
L-cytrulina
L-arginina
NOS
NO
O2
endothelium
K+
K+Ca
membrane
hyperpolarization
Ca2+
-
+
-
Guanylate cyclase
GTP
cGMP
iCa2+
vascular
smooth
muscle
cell
Protein
kinase G
Myosin light chains
dephosphorylation
vasorelaxation
vasorelaxation
cGMP)-specific
phosphodiesterase
type 5 (PDE5)
-
Viagra (Sildenafil), Pfizer

Vascular protective effects of nitric oxide

vasorelaxation

inhibition of proliferation of vascular smooth muscle cells

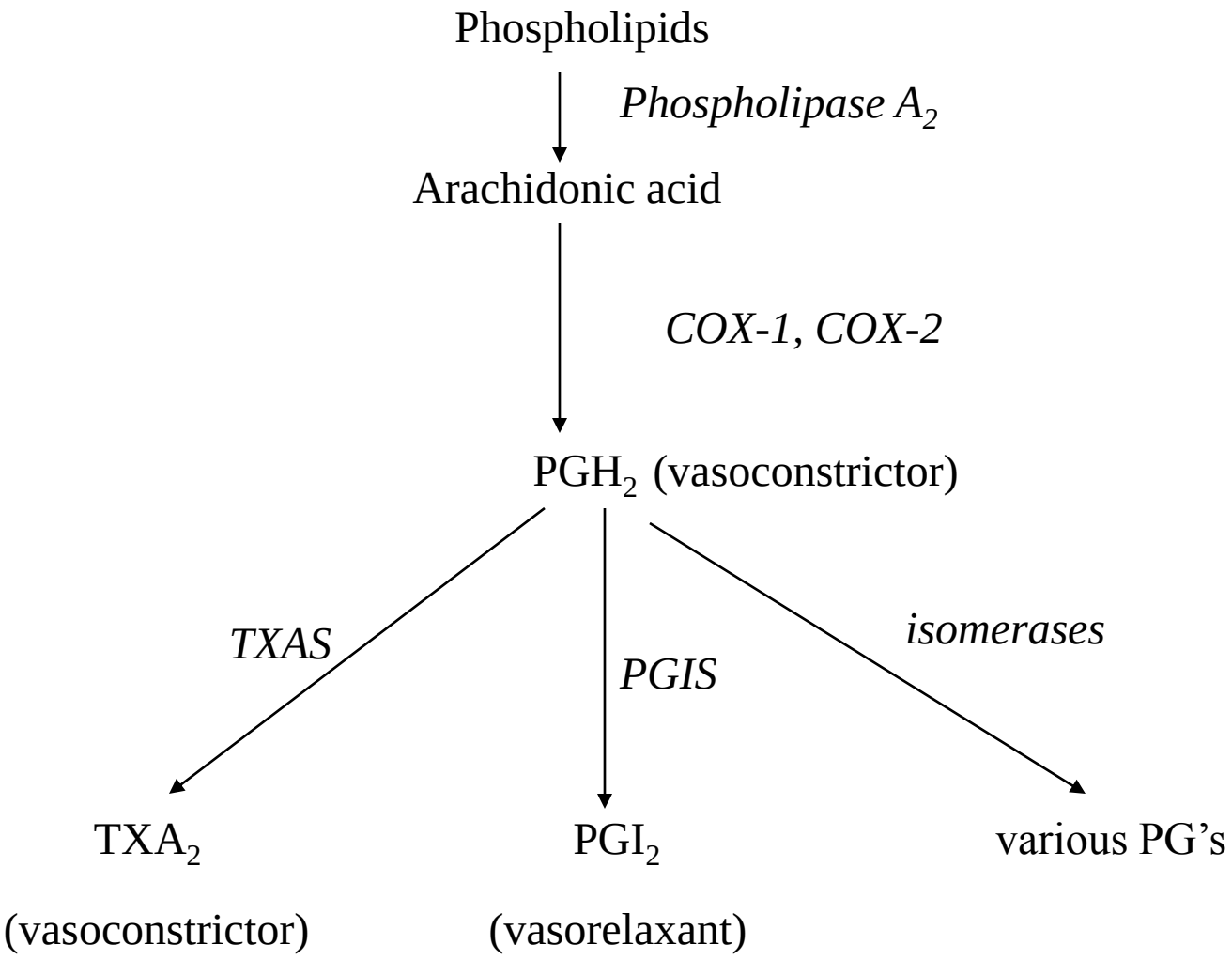
inhibition of platelet adhesion and aggregation

inhibition of monocyte adherence

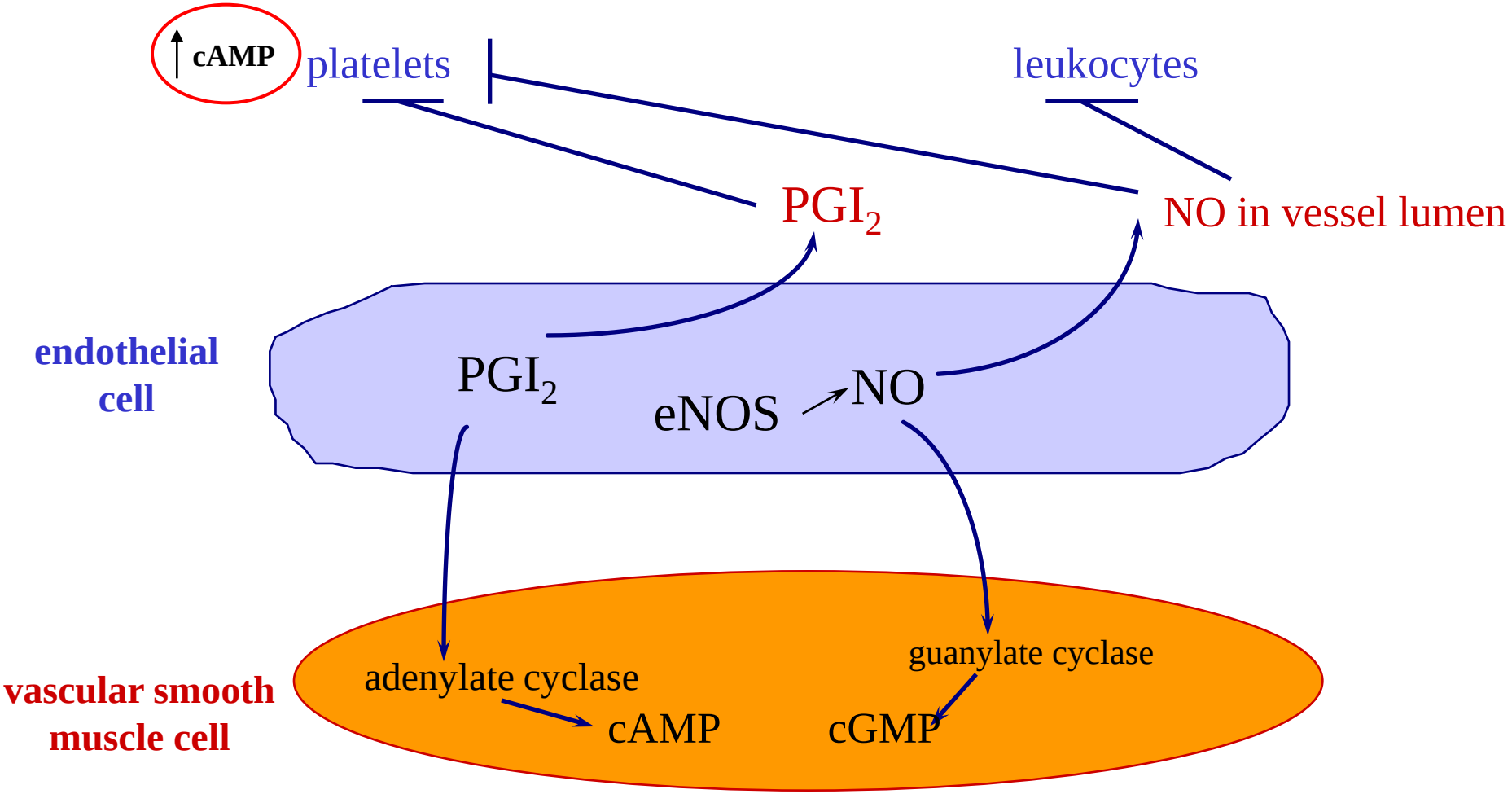
enhancement of endothelial cell survival and proliferation

Actions of PGI_2 are quite similar

Prostaglandin H synthase pathway



ROLE OF NITRIC OXIDE AND PROSTACYCLIN IN CARDIOVASCULAR SYSTEM



Discovery of nitric oxide

Robert Furchgot
Ferrid Murad
Luis Ignarro

Nobel prize - 1998

Prof. Ryszard J Gryglewski

Discovery of prostacyclin (1976)
(together with: Salvador Moncada)

First human appplication of prostacycline:
Prof.. Ryszard Gryglewski & Andrzej Szczeklik – 1977

VASCULAR ENDOTHELIUM

Normal endothelium

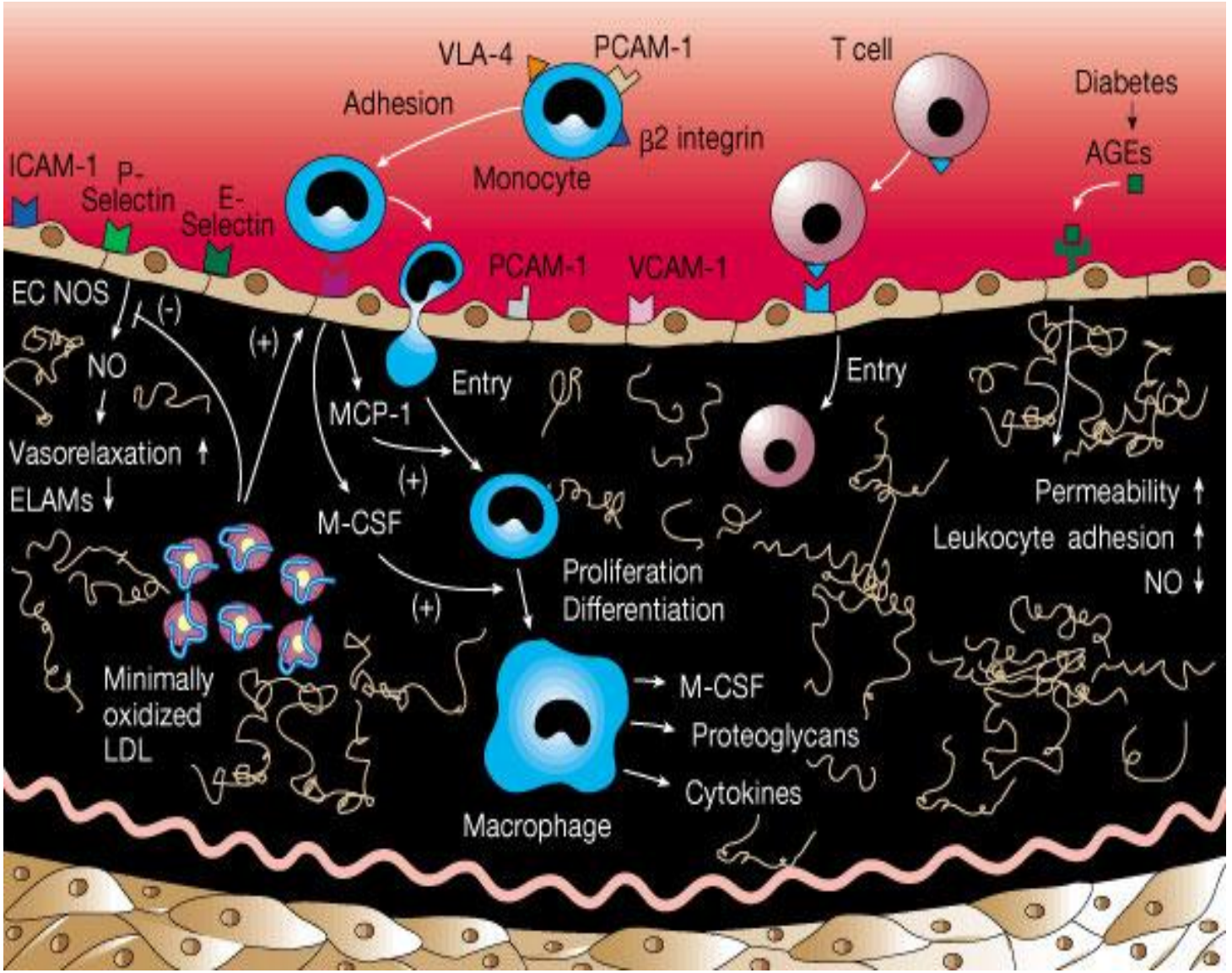
- permanent release of NO, PGI_2
- antithrombotic properties
- control VSMC relaxation and proliferation

Dysfunctional endothelium

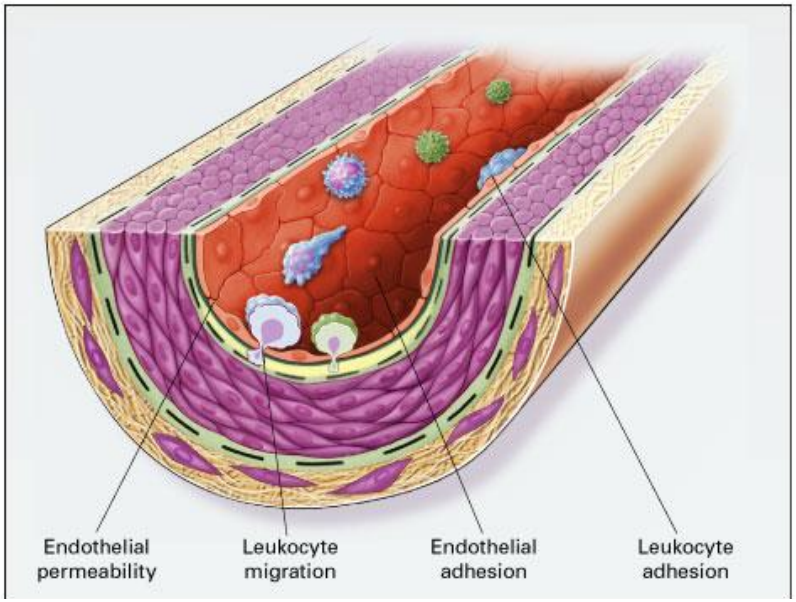
- impaired NO and PGI_2 synthesis
- inefficient prevention of thrombosis and VSMC proliferation

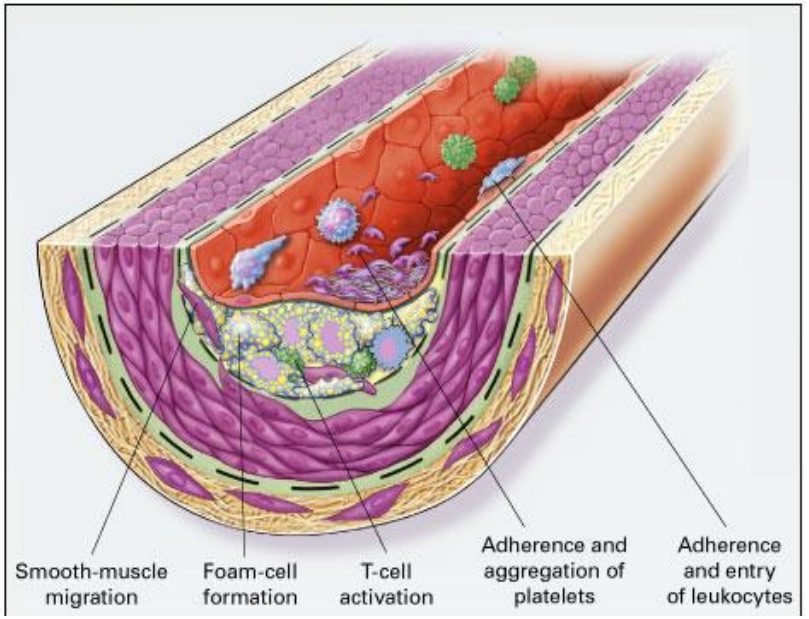
development of atherosclerotic plaque

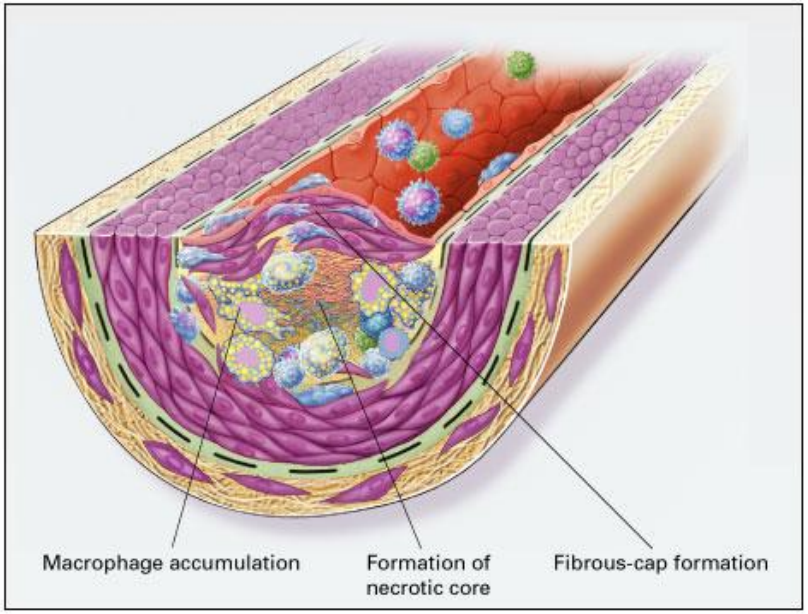
Development of inflammation in the arterial wall initiates atherosclerosis

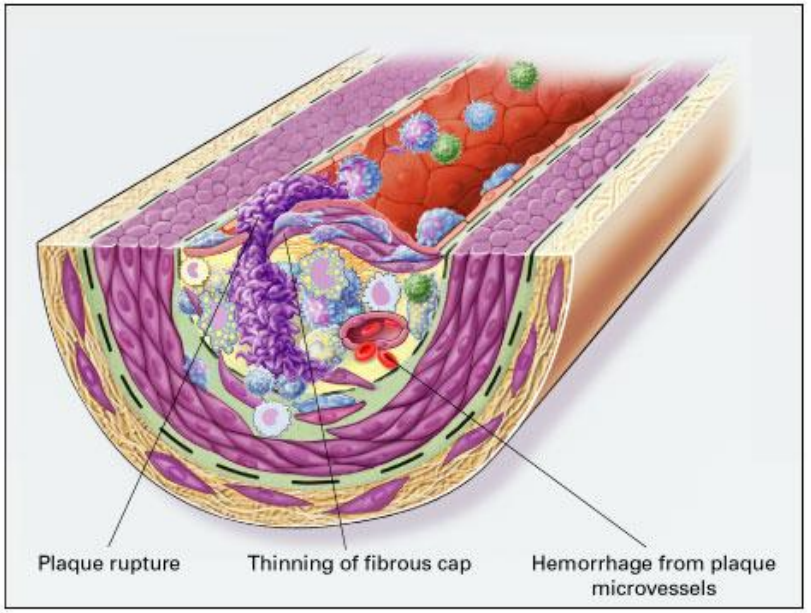


Progression of atherosclerosis





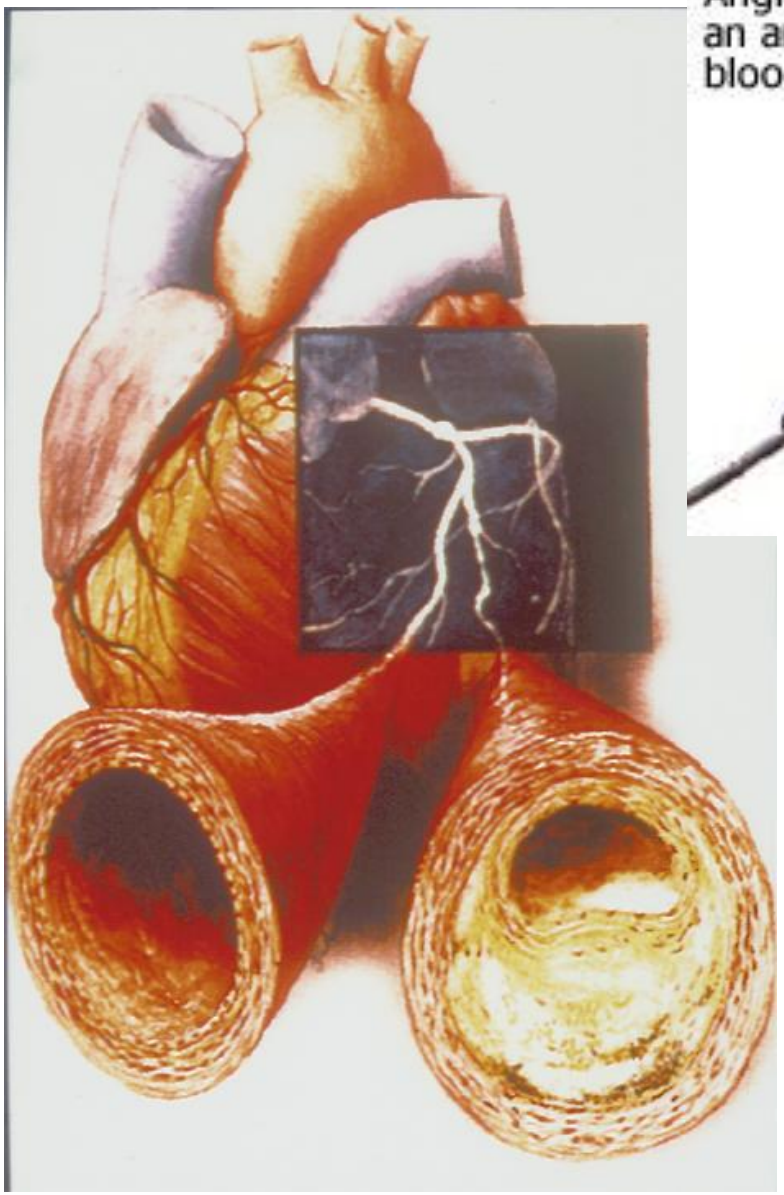


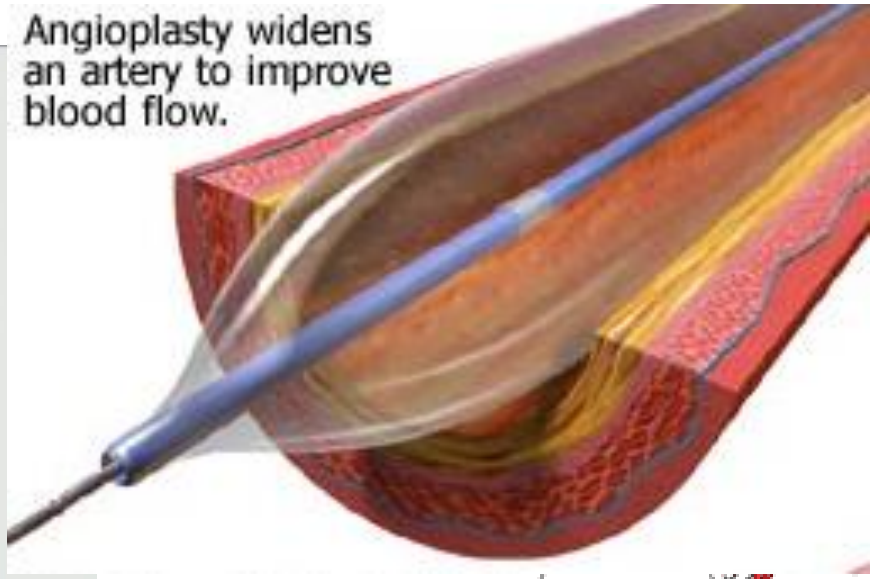


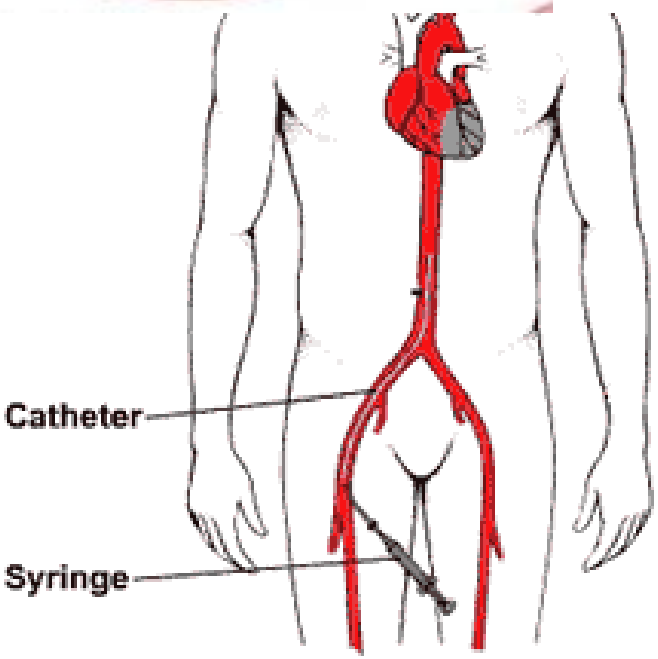
Ross, NEJM, 1999

Balloon angioplasty for prevention of vessel narrowing

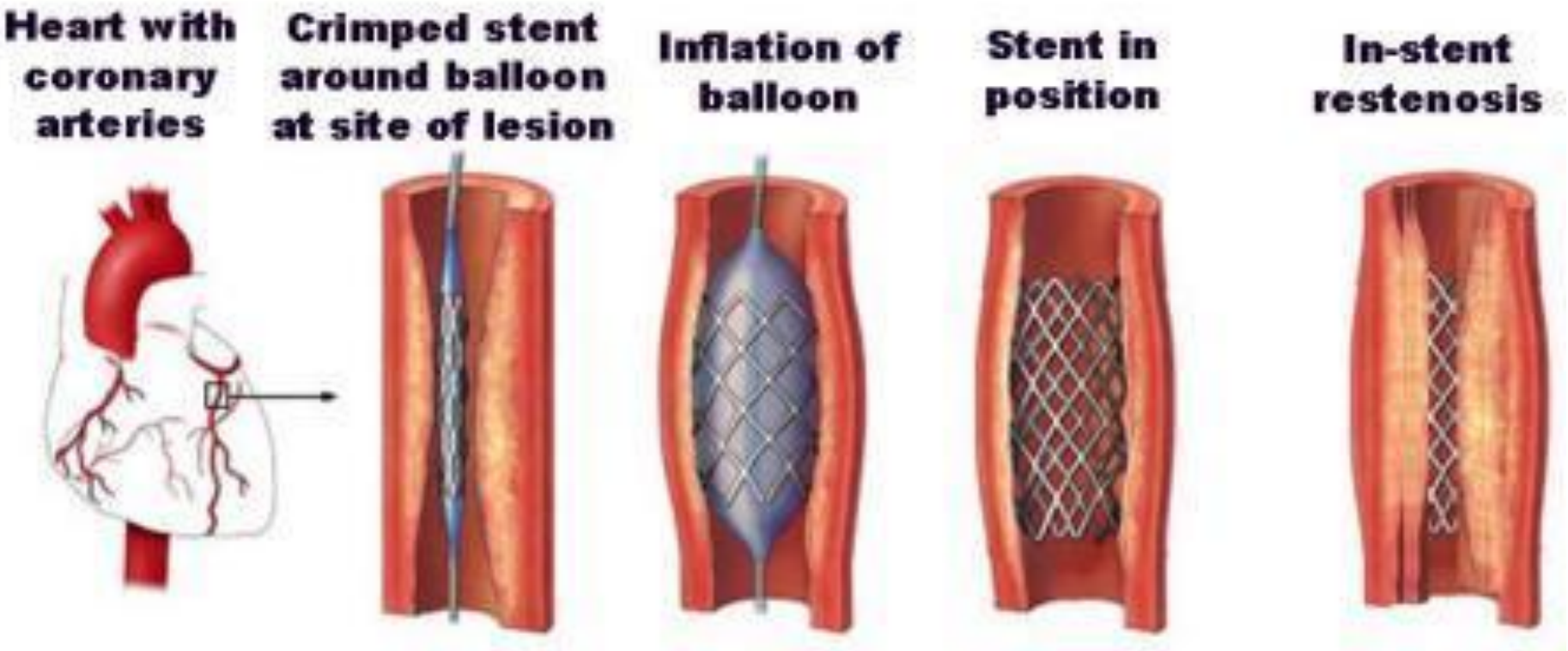
PTCA (Percutaneous Transluminal Coronary Angioplasty)



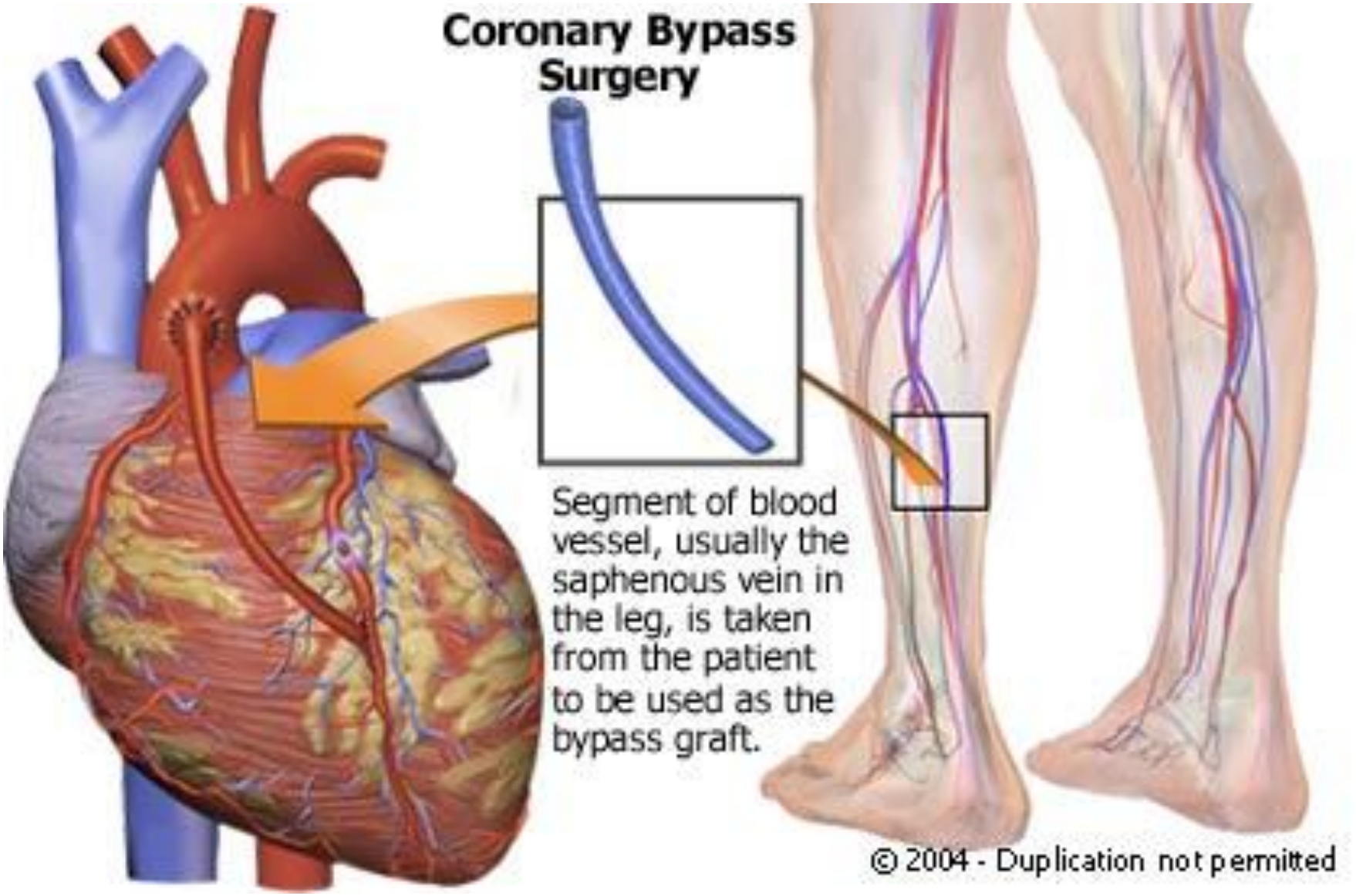




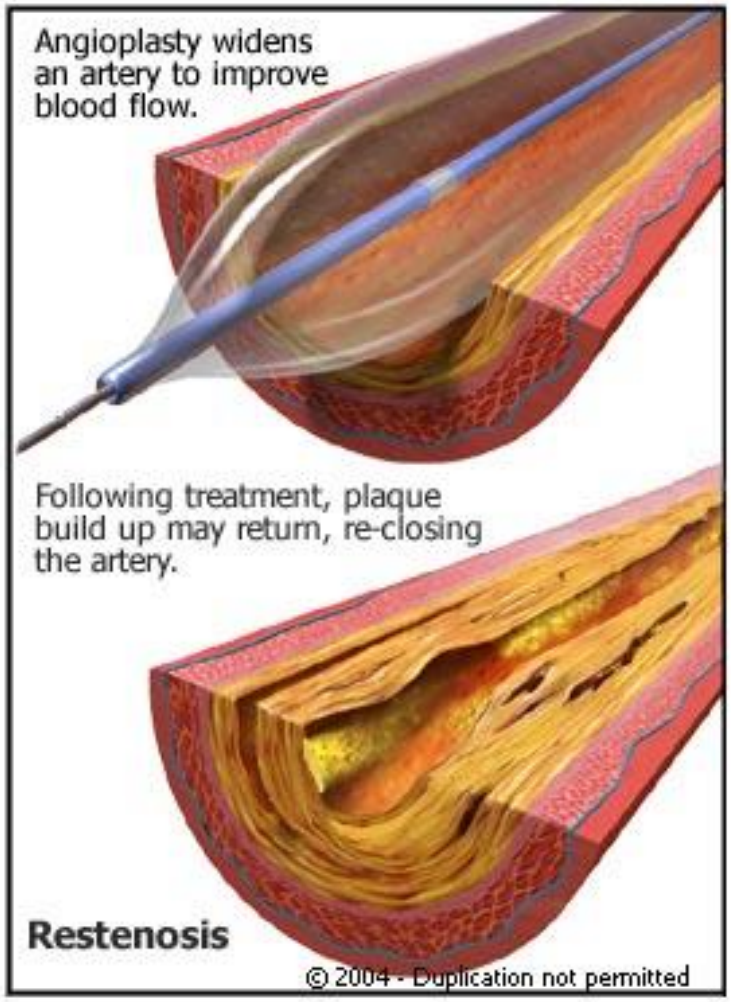
Treatmen of Atherosclerosis
STENT placement



Coronary bypass grafting (CABG)



Narowing of blood vessels after angioplasty or CABG

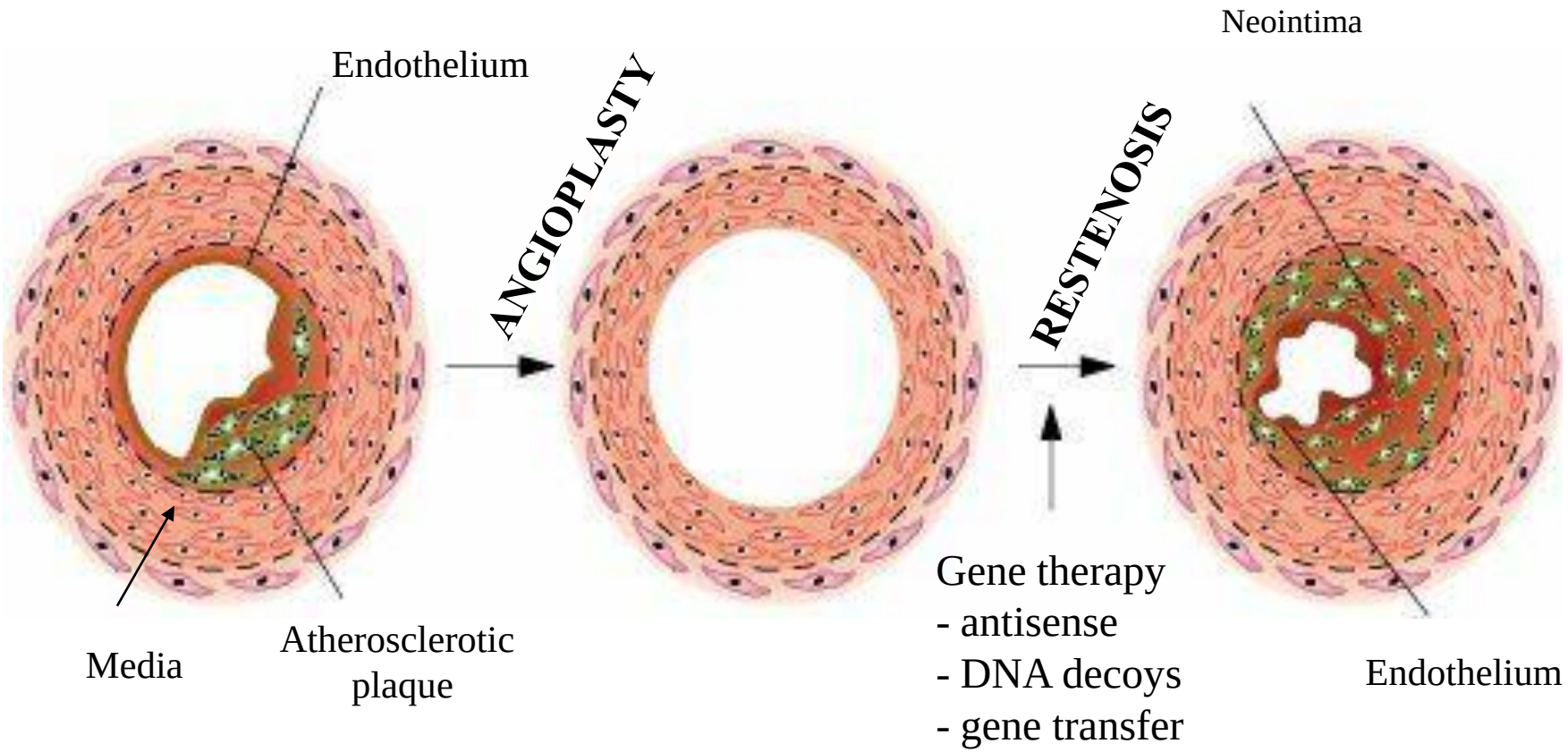


RESTENOSIS

narrowing occurs also in vessels used for by-pass grafting

- ***STENOSIS***

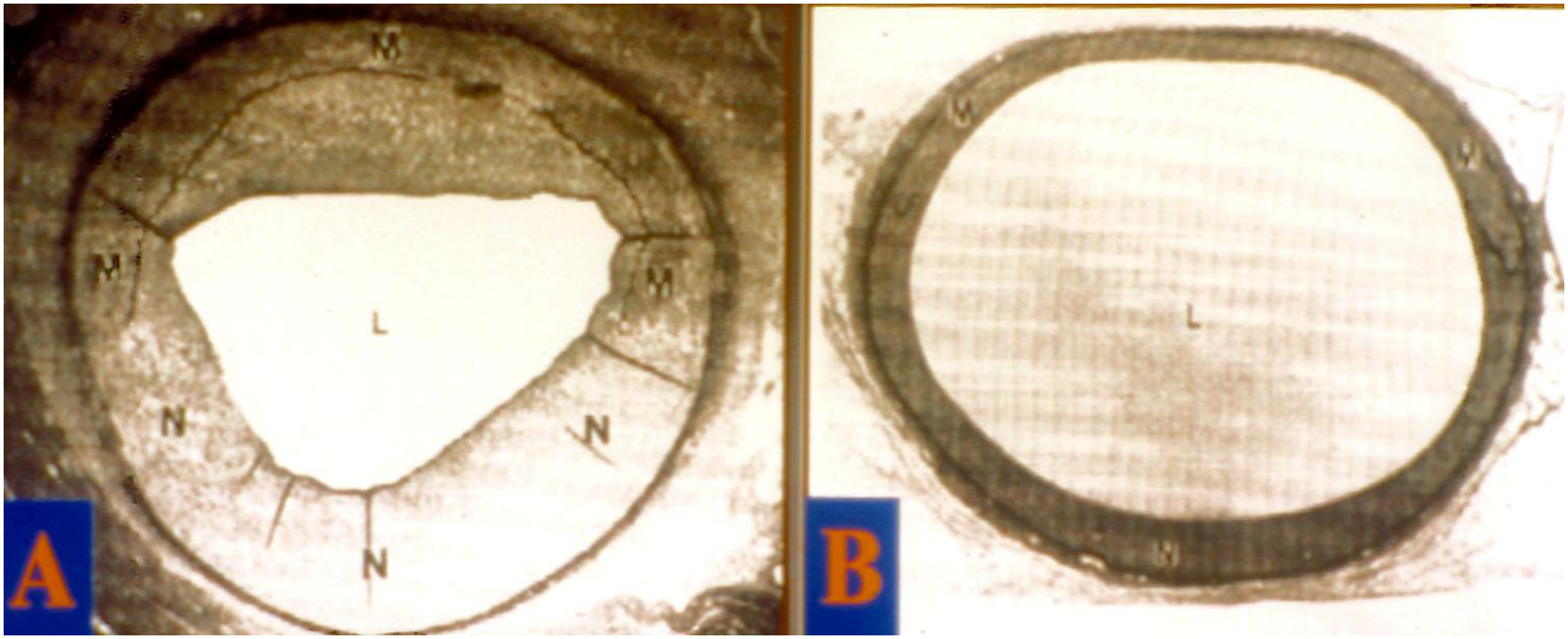
Gene therapy for treatment of neointima formation after balloon angioplasty



Gene therapy by overexpression of nitric oxide synthase

Transfer of endothelial nitric oxide synthase inhibits neointima formation in injured rat carotid artery

Vector: plasmid vector



Control gene (lacZ) transfected

eNOS transfected

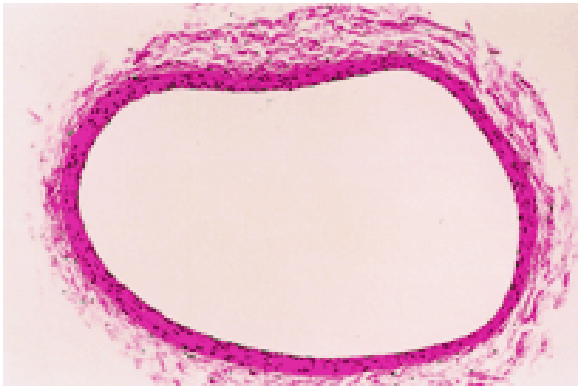
about 70% inhibition

Von der Leyen et al., PNAS, 1995

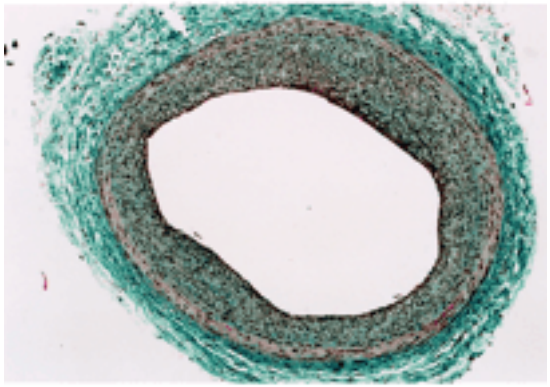
Stroke 1999 Feb;30(2):419-26

Gene transfer of human prostacyclin synthase prevents neointimal formation after carotid balloon injury in rats.

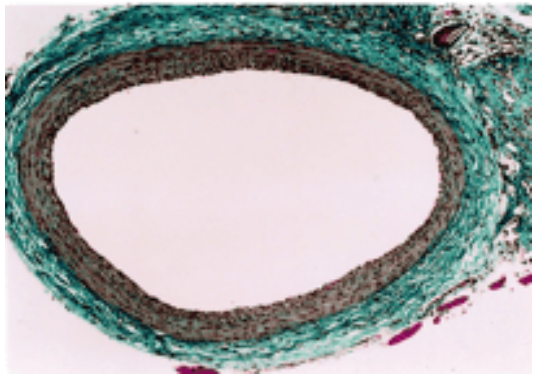
Todaka T, Yokoyama C, Yanamoto H, Hashimoto N, Nagata I, Tsukahara T, Hara S, Hatae T, Morishita R, Aoki M, Ogihara T, Kaneda Y, Tanabe T.



Control, not injured



Injured, control vector



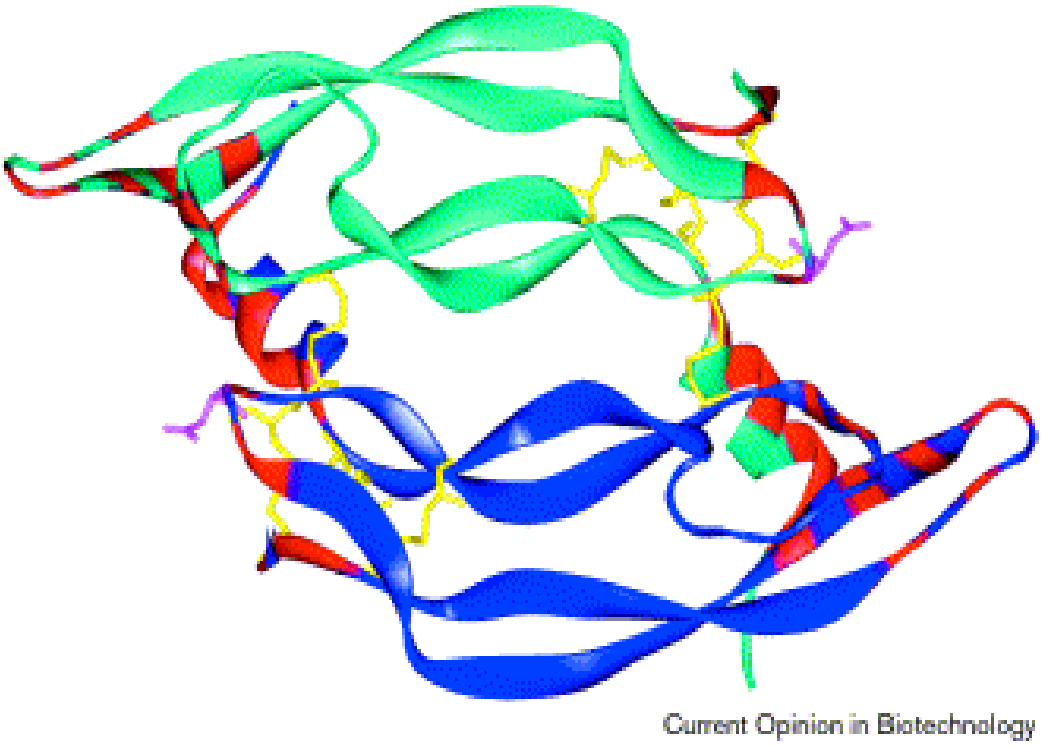
injured - PGIS transfection

Inhibition of neointimal hyperplasia may be achieved by transfer of:

- endothelial NO synthase (eNOS) gene,
- PGI synthase gene,
- cell cycle regulators (retinoblastoma, cyclin or cyclin-dependent kinase inhibitors, p53, growth arrest homeobox gene, fas ligand)
-antisense oligonucleotides to c-myb, c-myc, proliferating cell nuclear antigen,
- DNA decoys for transcription factors such as nuclear factor kappaB and E2F.

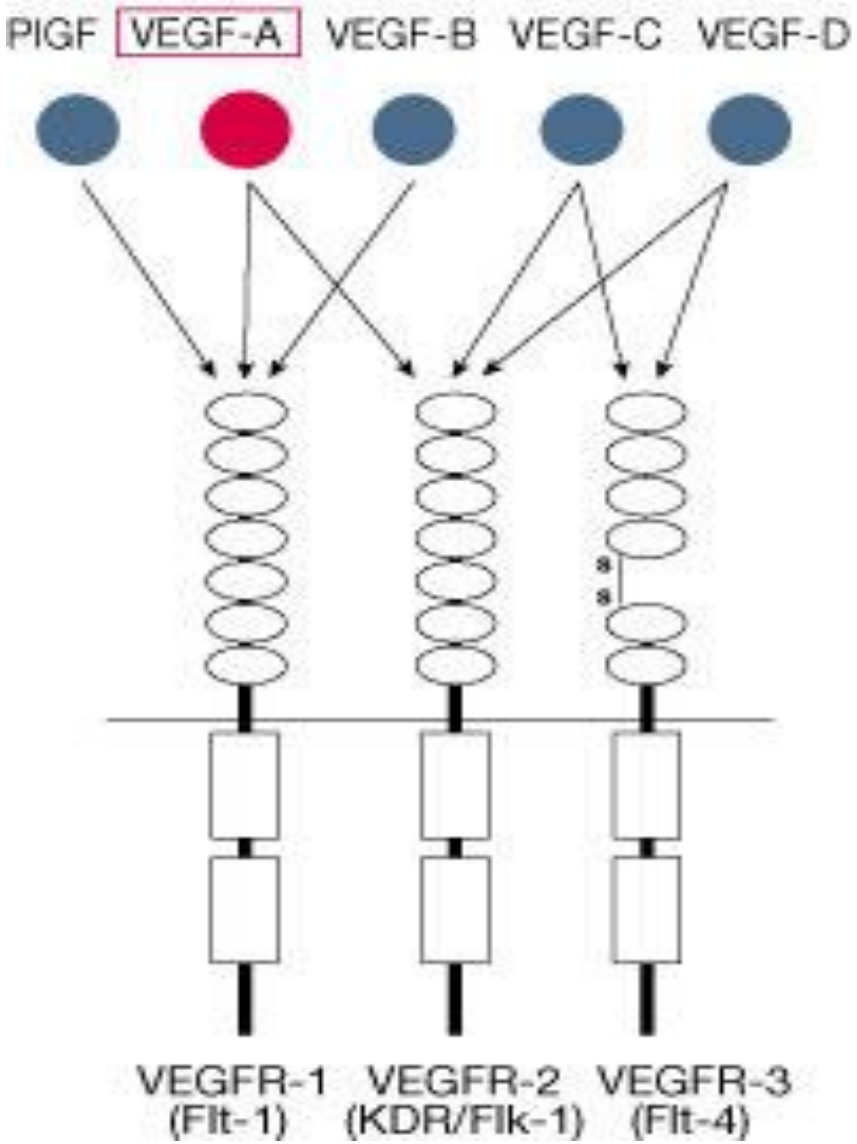
- but the best candidate seemed to be VEGF

Vascular endothelial growth factor (VEGF)



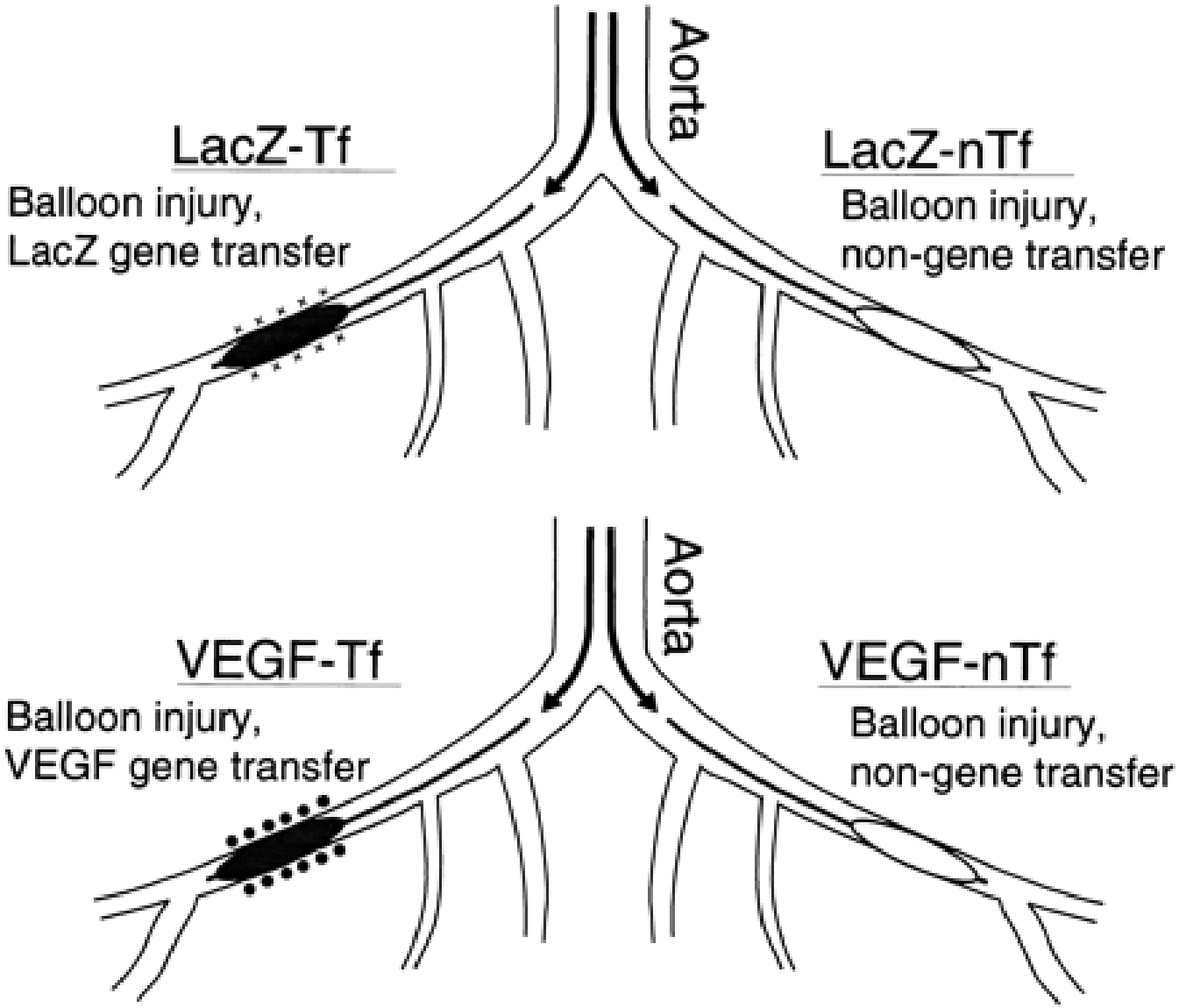
Growth factor that stimulates proliferation and migration of endothelial cells, and increases their survival

VEGF family of growth factors



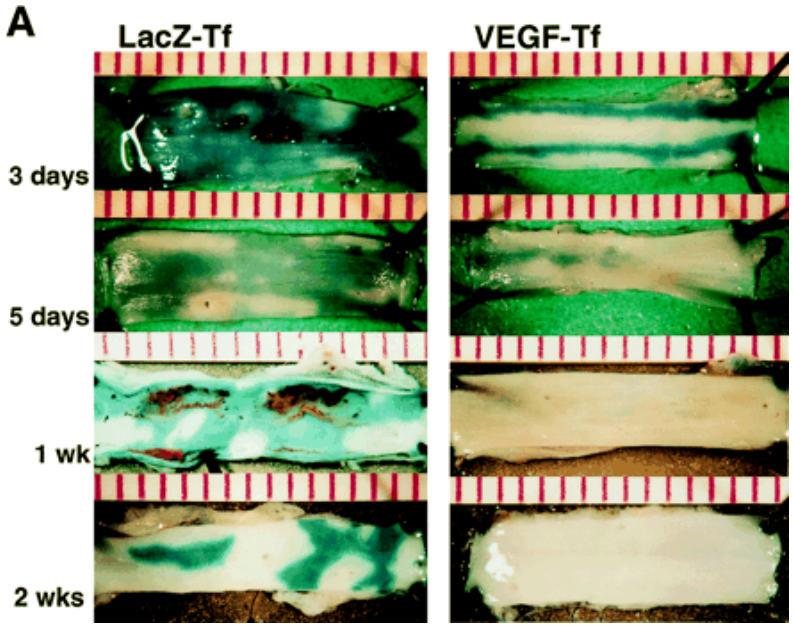
VEGF gene transfer for prevention of restenosis

Gene transfer of VEGF for inhibition of restenosis after balloon angioplasty

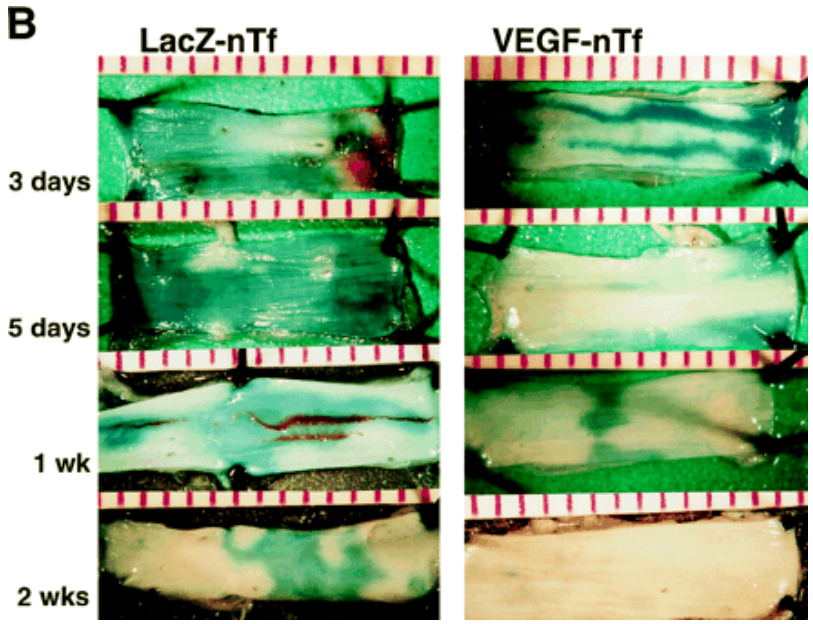


Asahara et al., Circulation, 1996

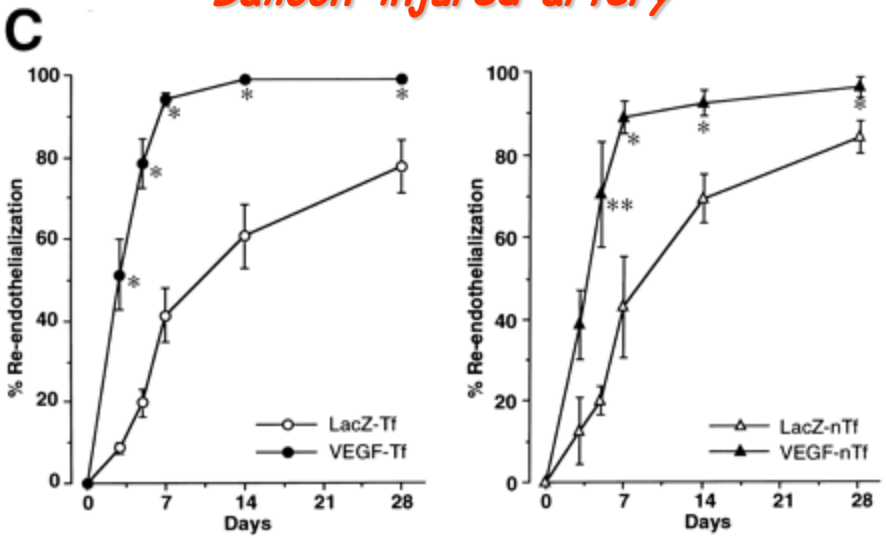
Gene transfer of VEGF for inhibition of restenosis after balloon angioplasty



Balloon-injured artery



contralateral artery



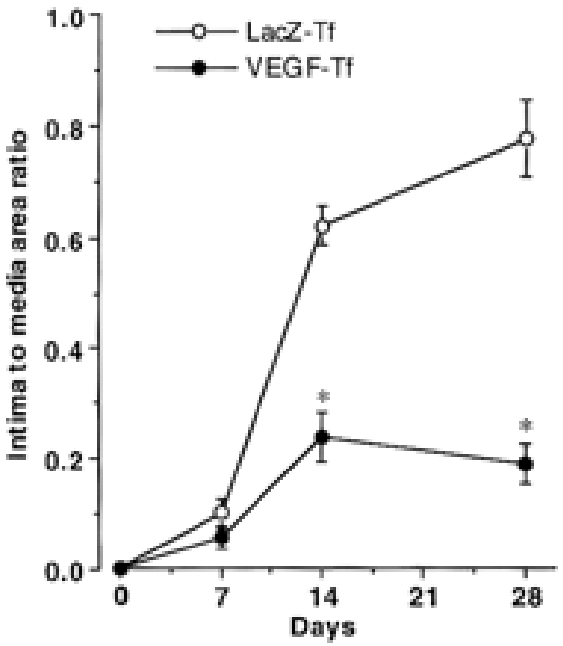
Balloon-injured artery

contralateral artery

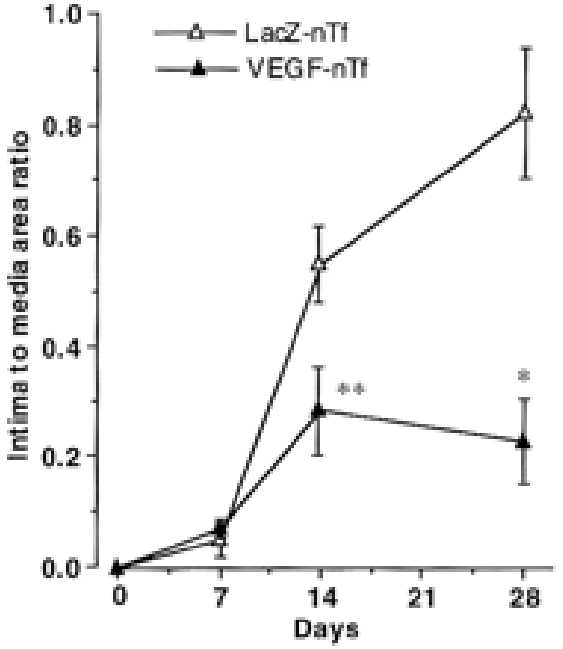
Representative macroscopic appearance of (A) balloon-injured, transfected arteries (LacZ-Tf and VEGF-Tf) and (B) contralateral balloon injured, nontransfected arteries (LacZ-nTf and VEGF-nTf) at 3 and 5 days and 1 and 2 weeks after transfection. rET area, not stained by Evans' blue dye, appears white. C, rET of injured transfected arteries (left) and injured nontransfected arteries (right). **P*<.01, ***P*<.05 vs LacZ-Tf or LacZ-nTF. VEGF-Tf: n=4, 4, 6, 8, and 8 at 3 and 5 days and 1, 2, and 4 weeks. VEGF-nTf: n=4, 4, 6, 8, and 8; LacZ-Tf: n=4, 4, 6, 6, and 5; and LacZ-nTf: n=3, 3, 5, 7, and 6, respectively.

Asahara et al., Circulation, 1996

Gene transfer of VEGF for inhibition of restenosis after balloon angioplasty

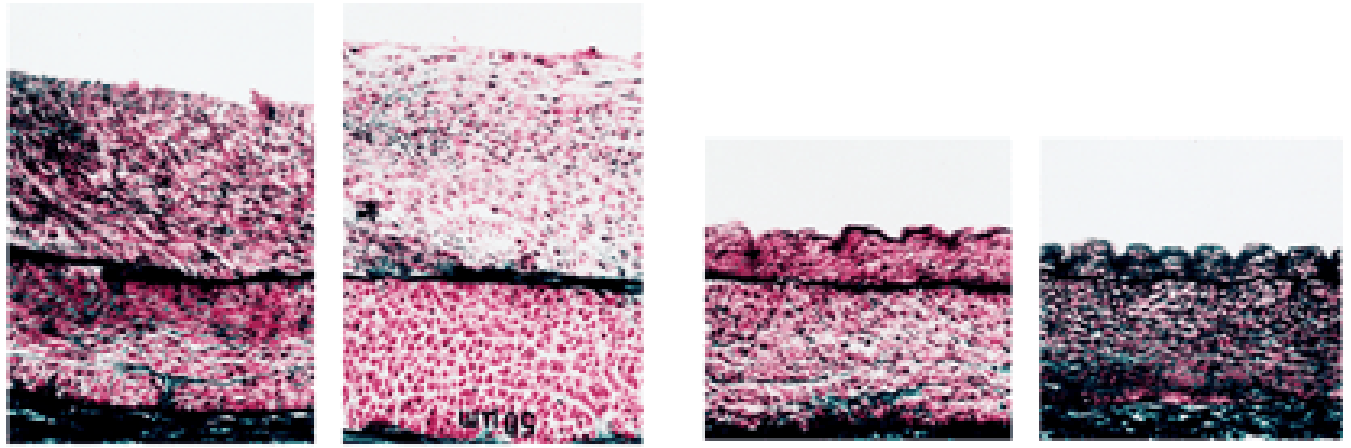


Balloon-injured artery



contralateral artery

C



LacZ-Tf (2wks) LacZ-Tf (4wks) VEGF-Tf (2wks) VEGF-Tf (4wks)

Asahara et al., Circulation, 1996

Marja Hedman, MD, PhD*; Juha Hartikainen, MD, PhD*; Mikko Syvänne, MD, PhD, FESC; Joachim Stjernvall, MD, PhD; Antti Hedman, MD, PhD; Antti Kivelä, MD; Esko Vanninen, MD, PhD; Hanna Mussalo, MD; Esa Kauppila, MD; Sakari Simula, MD, PhD; Outi Närvänen, PhD; Arto Rantala, MD; Keijo Peuhkurinen, MD, PhD, FESC; Markku S. Nieminen, MD, PhD, FESC; Markku Laakso, MD, PhD; Seppo Ylä-Herttuala, MD, PhD, FESC

Safety and Feasibility of Catheter-Based Local Intracoronary Vascular Endothelial Growth Factor Gene Transfer in the Prevention of Postangioplasty and In-Stent Restenosis and in the Treatment of Chronic Myocardial Ischemia

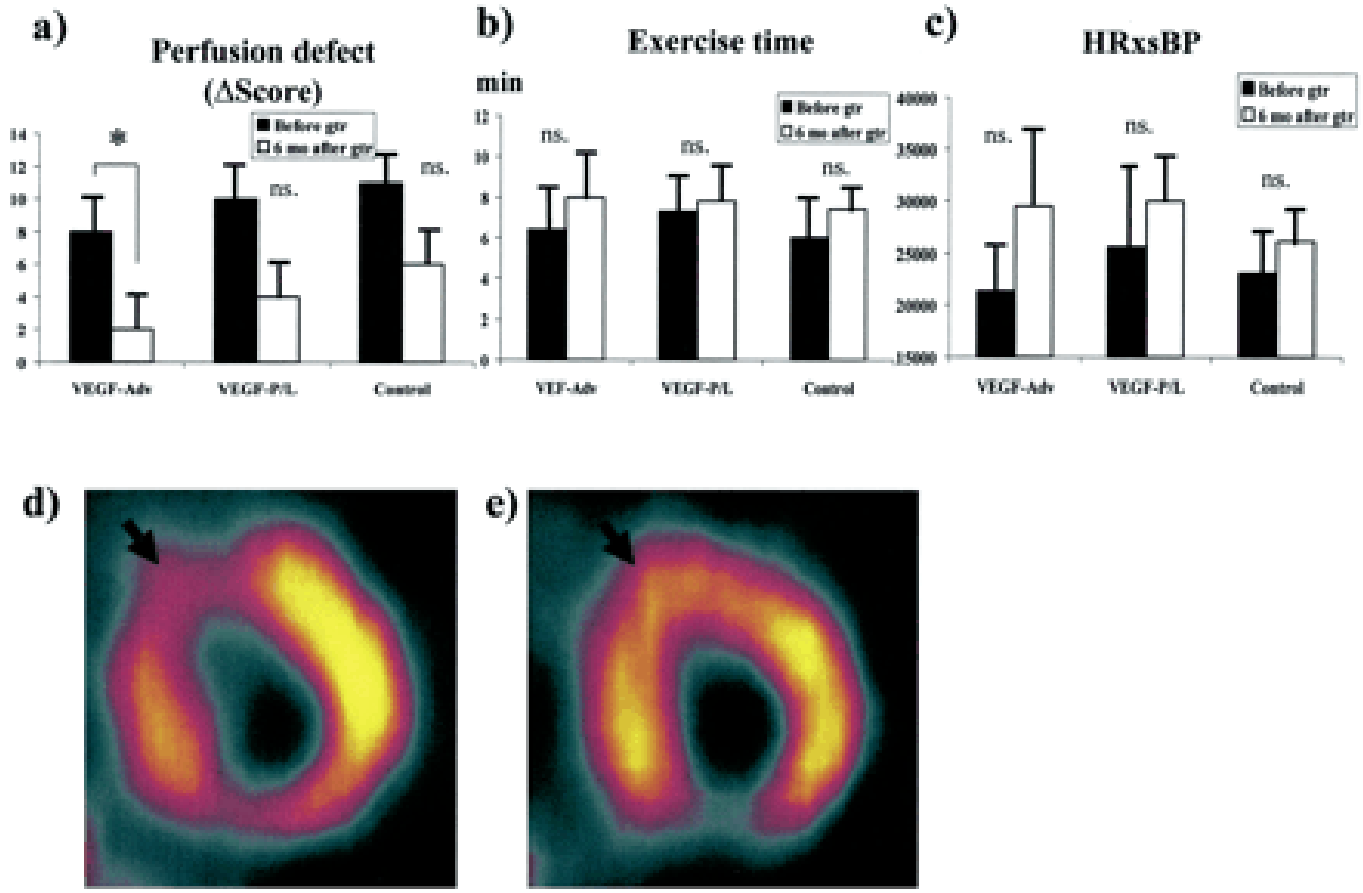
Phase II Results of the Kuopio Angiogenesis Trial (KAT)

Circulation. 2003;107:2677

Double-blind, randomized study

1. Ringer saline
2. Plasmid
3. Adenoviral vectors

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. Gtr indicates gene transfer.

Hedman M, et al.. Circulation. 2003;107:2677

Currently, the treatments approaches decreased significantly the rate of restenosis

-drug –eluting stents (sirolimus/rapamycin paclitaxel)

Angiogenic gene therapy

ANGIOGENESIS

the formation of new blood vessels by a process of sprouting from preexisting ones

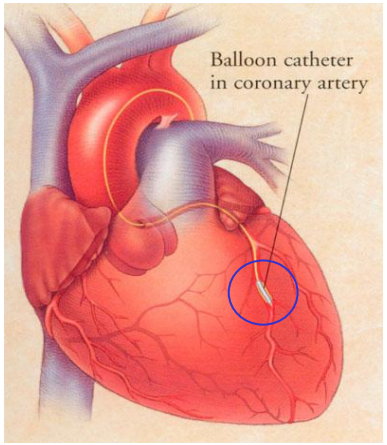
- activation of endothelial cells (EC)
- dissolution of the matrix underlying the endothelium
- migration, reattachment (adhesion) and proliferation of EC
- migration and proliferation of VSMC in vessels larger than capillaries
- formation of a new three-dimensional tube

Neovascularization vs therapeutic angiogenesis

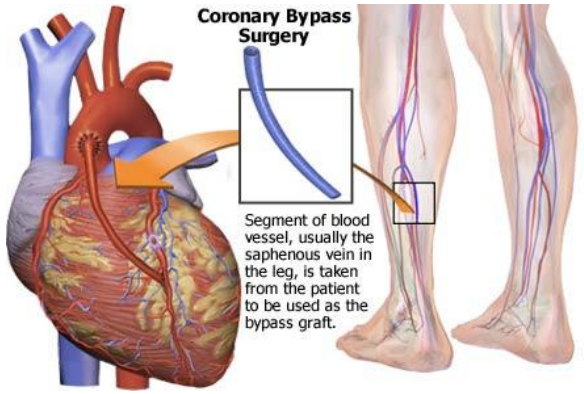
- Therapeutic angiogenesis describes the method of improving the blood flow to the ischemic tissue by the induction of postnatal neovascularization by angiogenic agents delivery

- This form of **gene therapy**, has emerged as a method for the treatment of patients who are not candidates for more conventional methods of revascularization (PTCA, CABG) due to inoperable disease or very small blood vessels

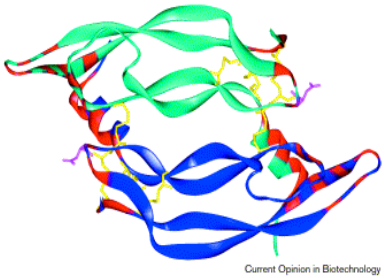
Percutaneous Transluminal Coronary Angioplasty (PTCA)

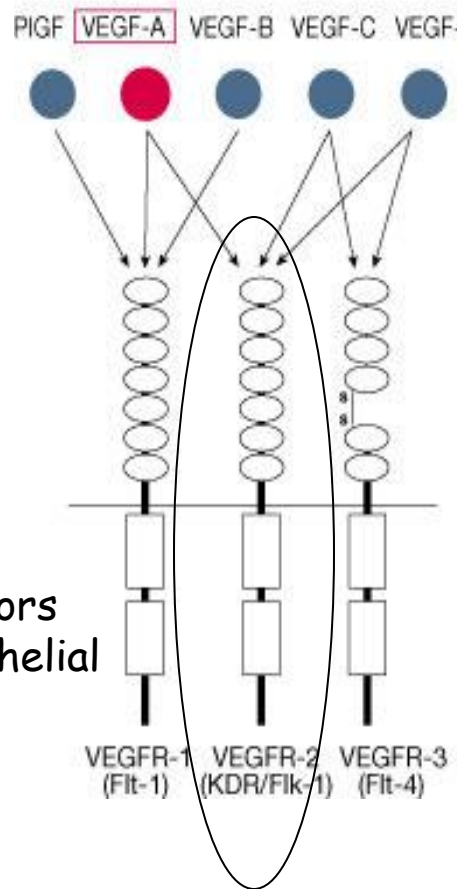


Coronary Artery Bypass Grafting (CABG)

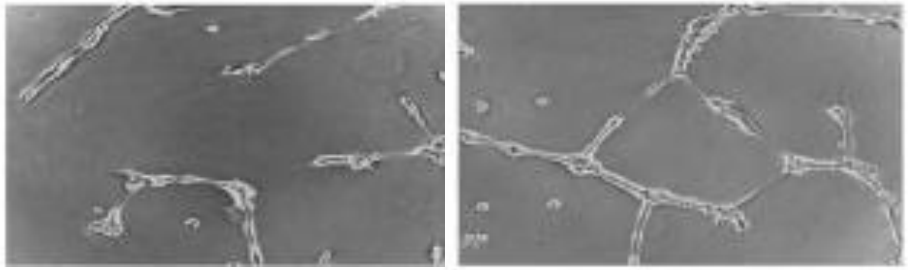


VEGF-A – a major angiogenic mediator

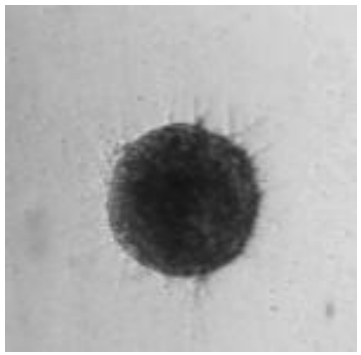


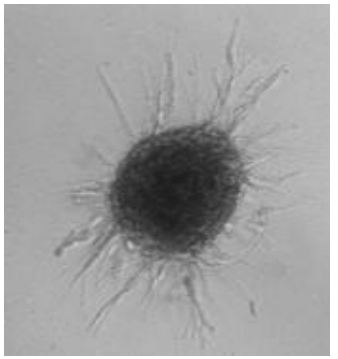


Receptors on endothelial cells



Matrigel assay

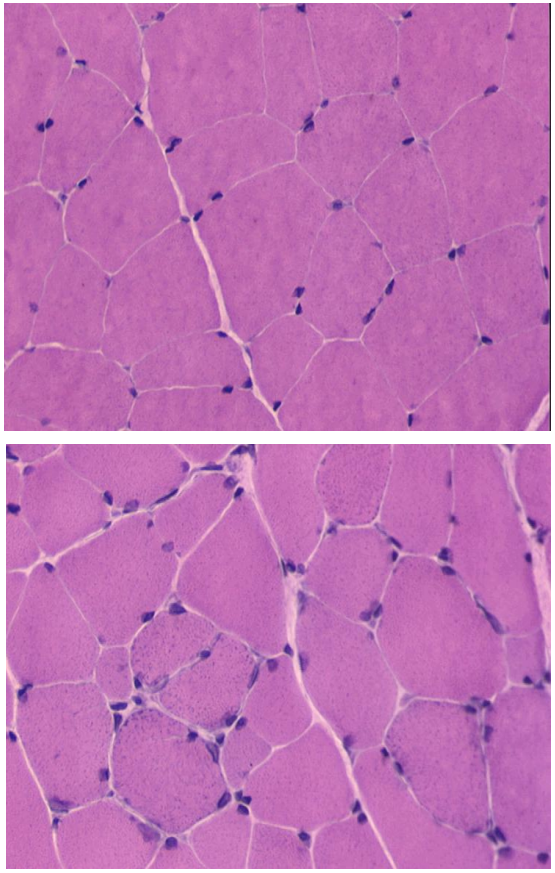




spheroid assay

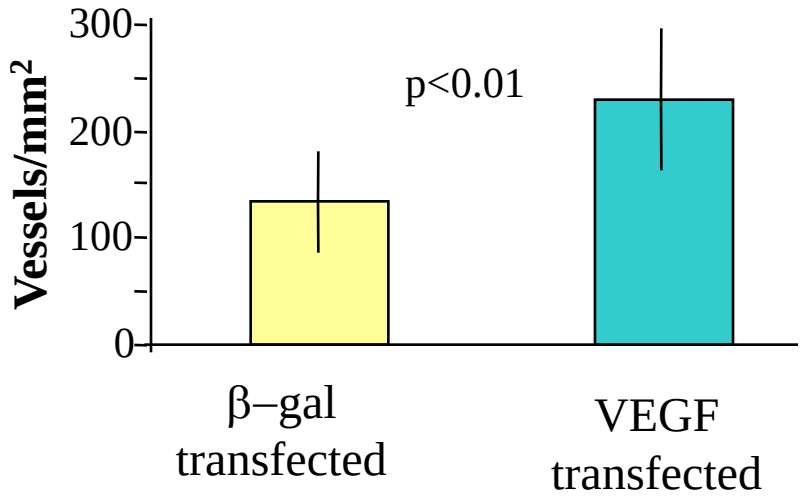
control **VEGF**

Naked VEGF gene transfer increases the number of microvessels in the ischemic rabbit skeletal muscles



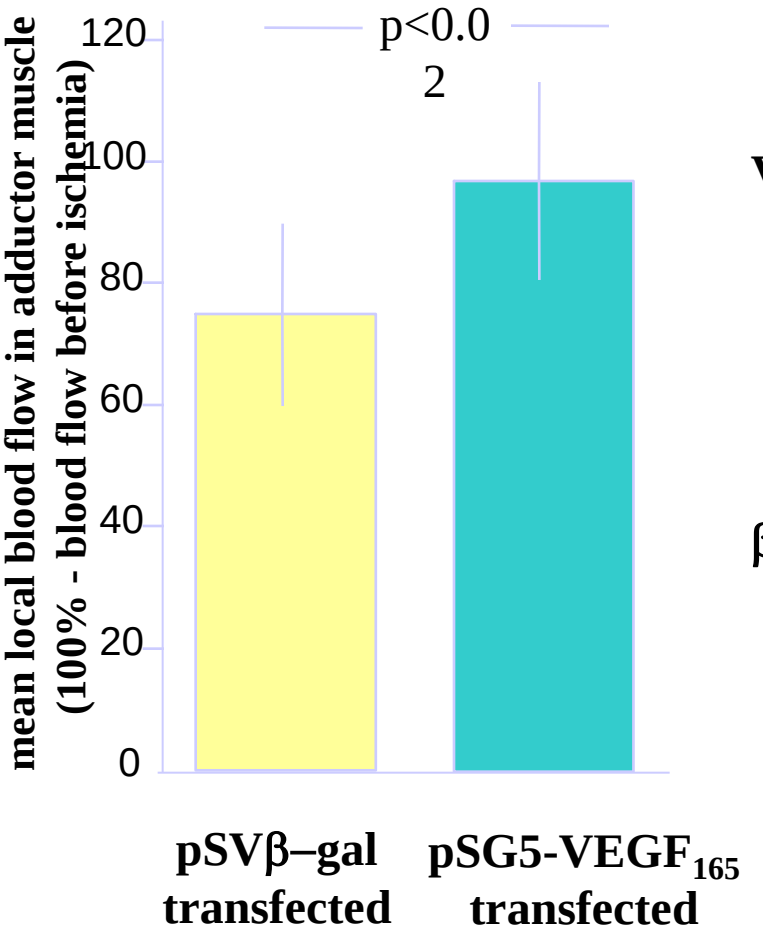
alkaline phosphatase-positive microvessels in muscle transfected with control plasmid

microvessels in muscle transfected with VEGF plasmid

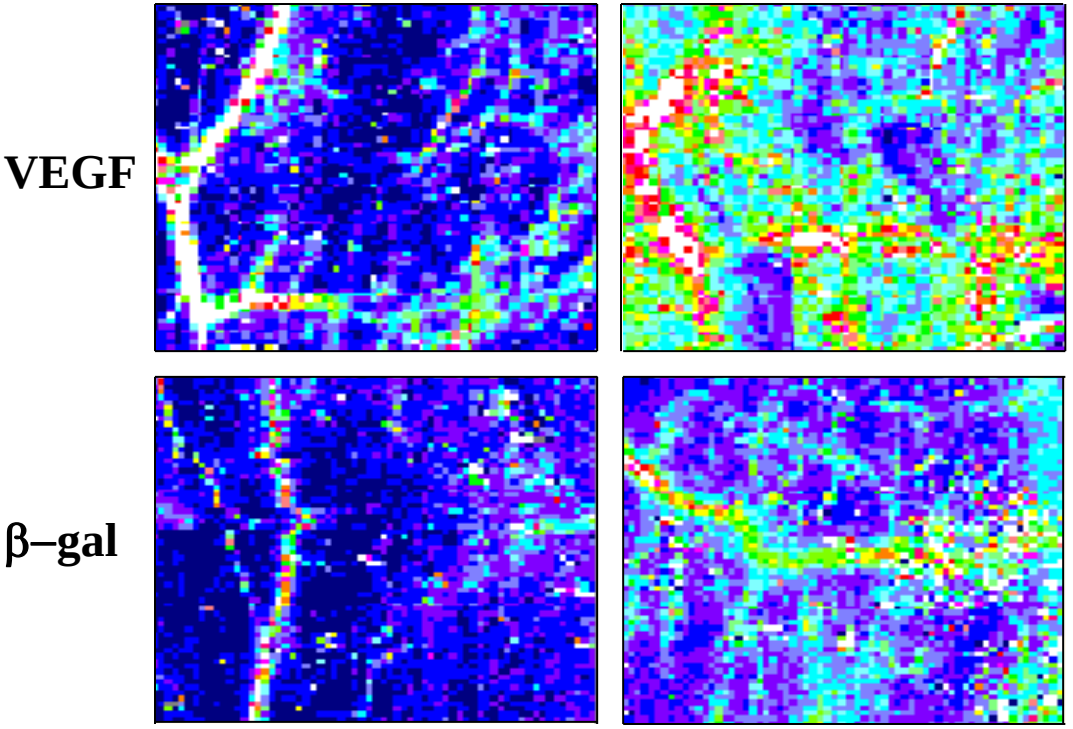


Dulak et al., Eur Surgery 2002, 34: 105-110; Józkowicz et al., Int J Artif Organs, 2003: 26: 161-169

$VEGF_{165}$ gene transfer improves blood flow in the rabbit adductor muscle



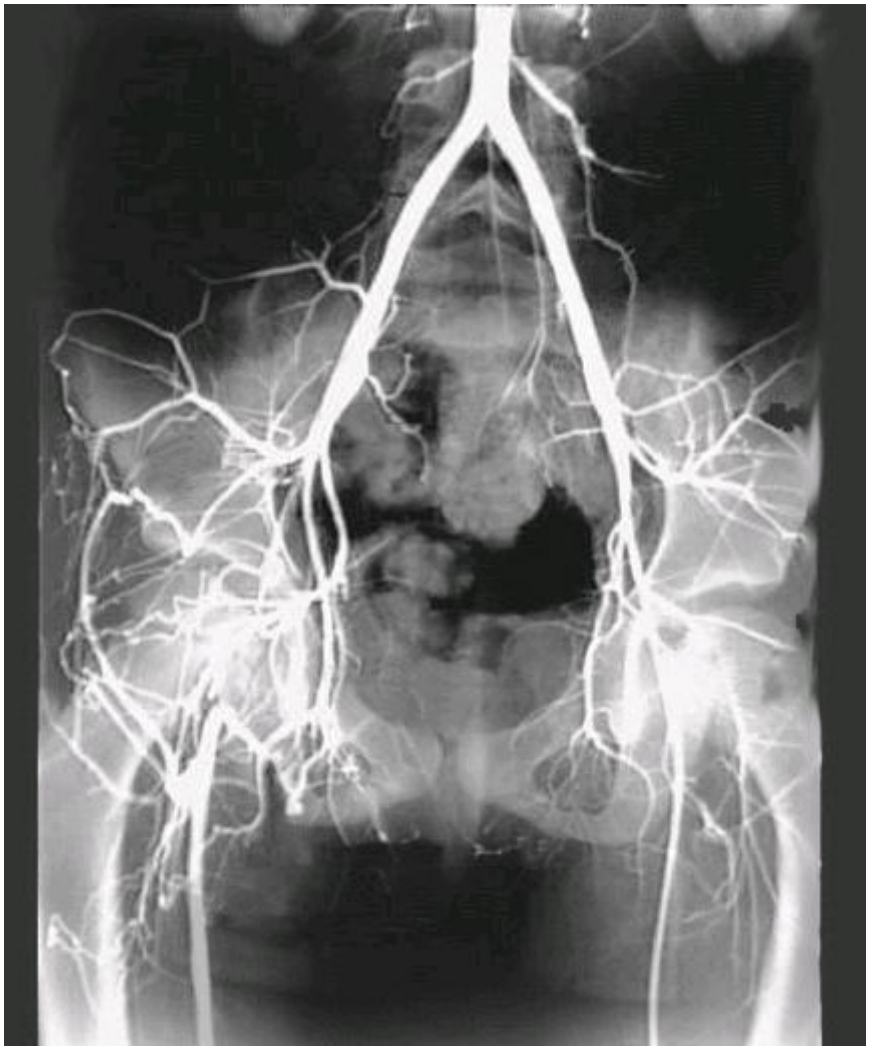
Blood flow in ischemic adductor muscles

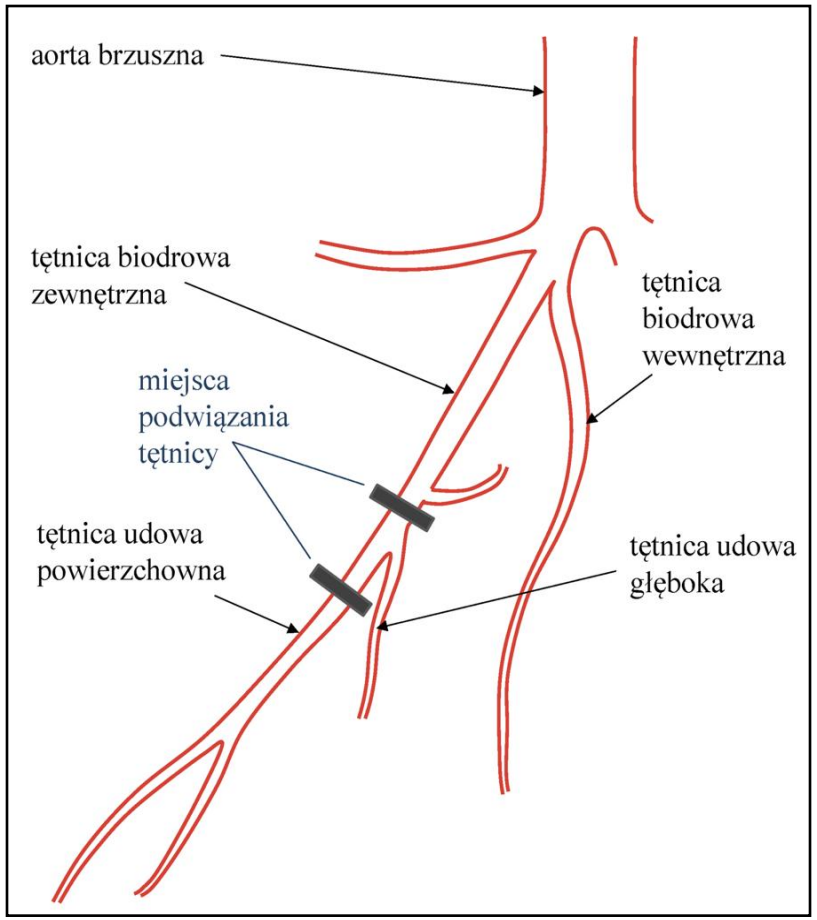


Dulak et al., Eur Surgery 2002, 34: 105-110; Józkowicz et al., Int J Artif Organs, 2003: 26: 161-169

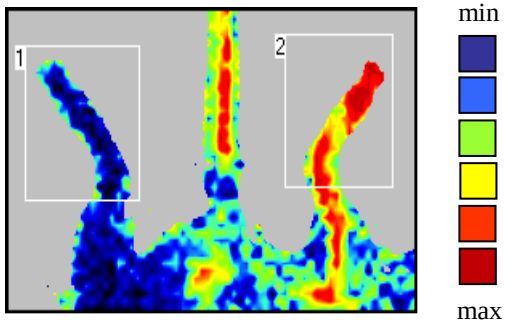
Mouse model of hind-limb ischemia

Surgical ligation of the femoral artery)

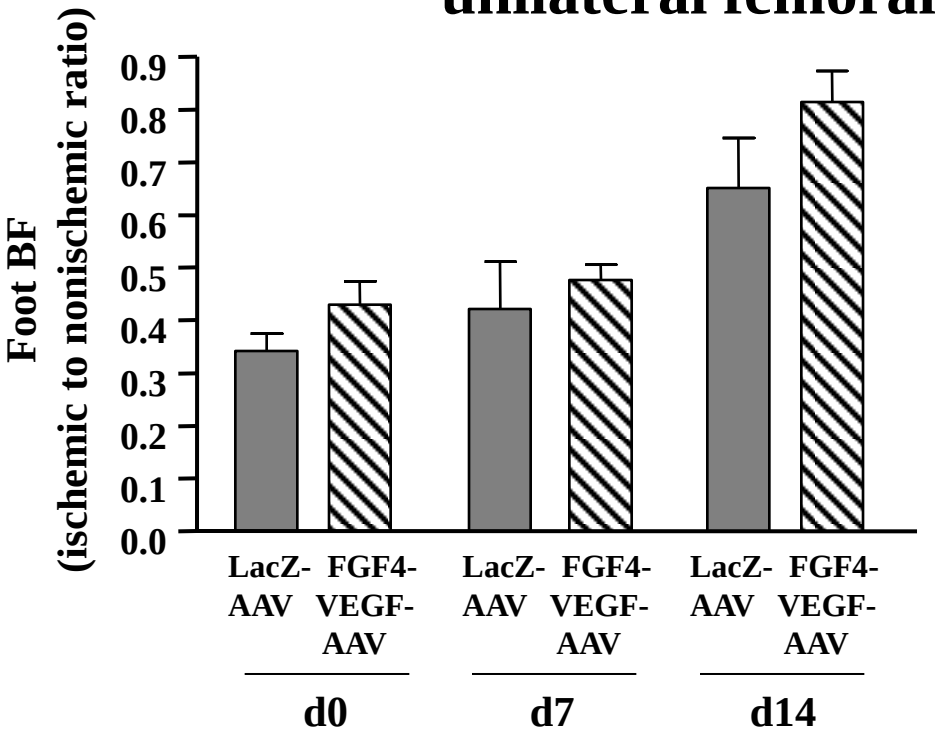




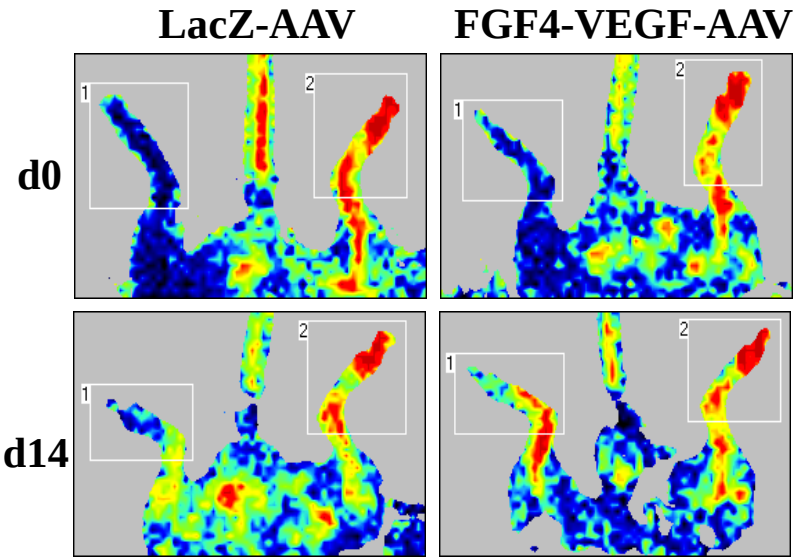
Laser Doppler perfusion measurement



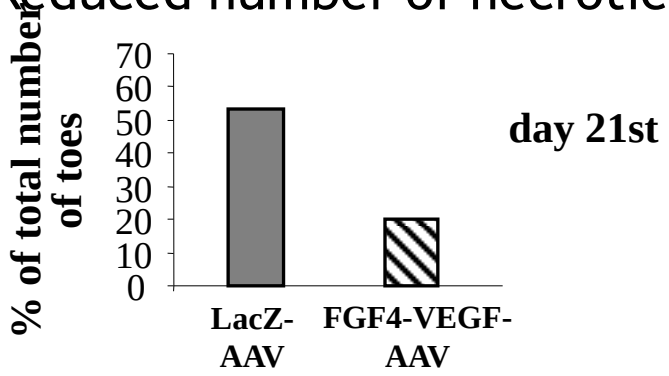
Recovery of hind-limb blood flow in C57Bl mice after unilateral femoral artery occlusion

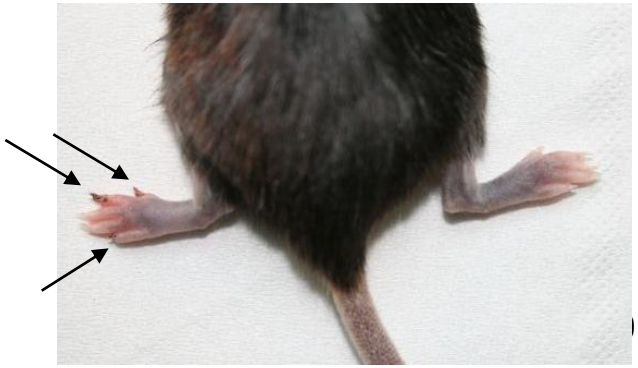


Laser Doppler perfusion measurement



Reduced number of necrotic toes





Jaźwa et al., in preparation

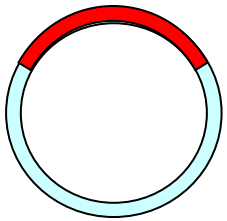
Gene transfer of VEGF can stimulate the formation of new blood vessels in ischemic limbs

Critical limb ischemia

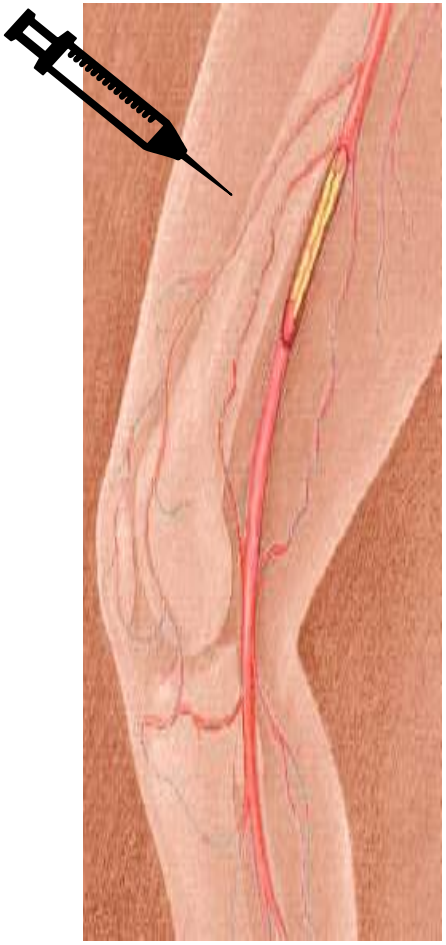
CRITICAL LIMB ISCHEMIA (CLI) is a disease manifested by sharply diminished blood flow to the legs; it is the most common cause of nontraumatic amputation in diabetes. The condition is responsible for 70% of the 150 lower limb amputations per million population (Eskelinen *et al*., 2004). Although a combination of neuropathy, obstructive macrovascular disease, and/or microvascular changes is usually pivotal in the development of the diabetic foot, the contribution of microvascular occlusions is predominant in the diabetic subgroup with CLI and is not accessible for surgical revascularization. Amputation is unavoidable in 0.7 per 10,000 patients with diabetes mellitus (da Silva *et al*., 1996; Holstein *et al*., 2000). Among CLI patients who have already had all possible surgical revascularization done, amputation is inevitable in approximately half (da Silva *et al*., 1996; Klevsgard *et al*., 2001). The median survival of patients with CLI is approximately 3 years (ICAI Group, 1997; Cheng *et al*., 2000). The quality of life during this period is limited (Albers *et al*., 1992).

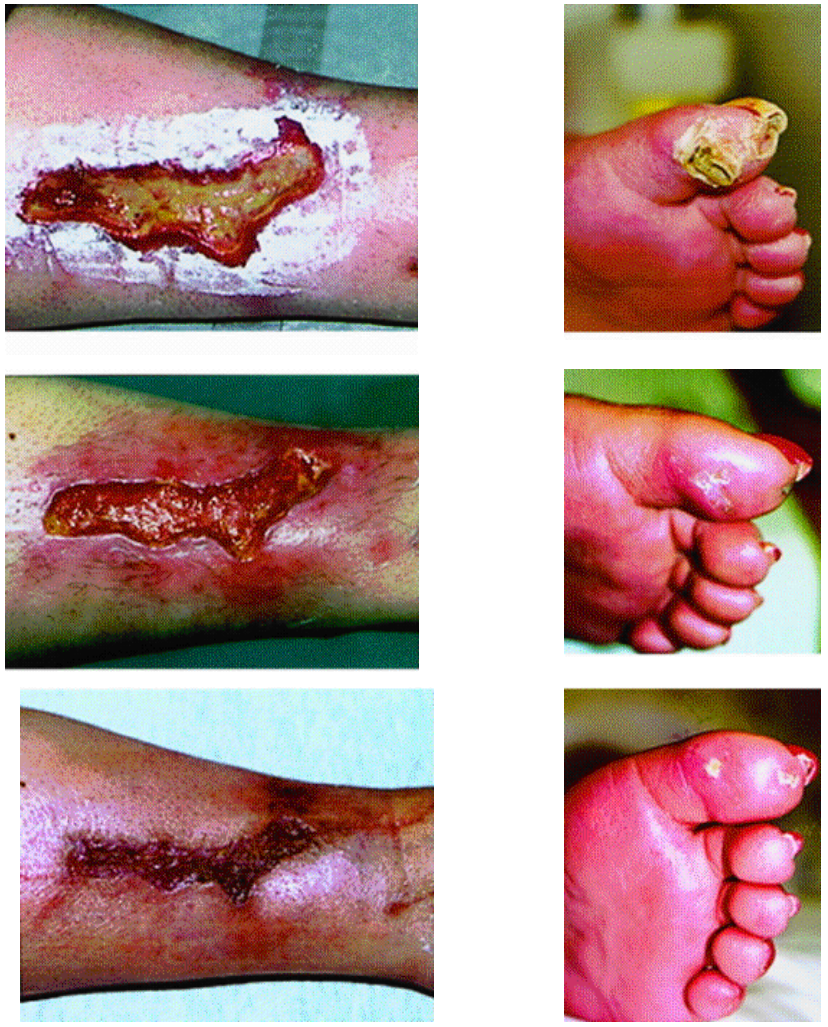
Gene transfer of VEGF in critical leg ischemia

Before VEGF gene therapy



pVEGF

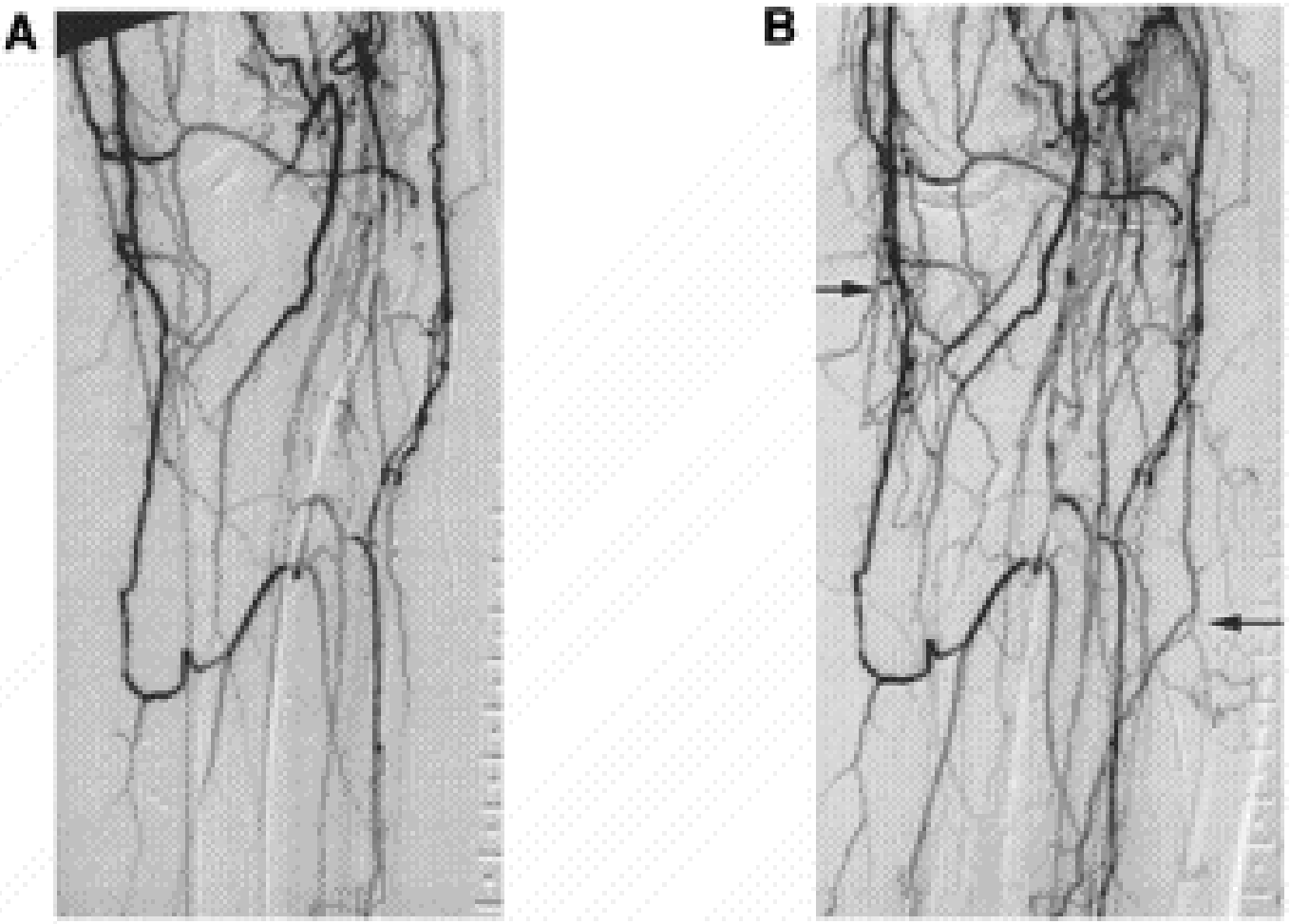




After VEGF gene therapy

Baumgartner i wsp., Circulation, 1998

Newly visible collateral vessels at calf level 8 weeks after phVEGF$_{165}$ gene transfer. Luminal diameter of newly visible vessels ranged from 200 to >800 μm (arrow); most were closer to 200 μm, and these frequently appeared as a blush of innumerable collaterals.



Baumgartner I et al., Circulation, 1998

Clinical trials in cardiovascular gene therapy

Majority of studies: negative results

Experimental studies have established the proof-of-principle that gene transfer to the cardiovascular system can achieve therapeutic effects. First human clinical trials provided initial evidence of feasibility and safety of cardiovascular gene therapy. **However, phase II/III clinical trials have so far been rather disappointing** and one of the major problems in cardiovascular gene therapy has been the inability to verify gene expression in the target tissue. New imaging techniques could significantly contribute to the development of better gene therapeutic approaches.

Are plasmid vectors effective in stimulation of angiogenesis in ischemic myocardium?

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CLINICAL RESEARCH **Clinical Trial**

Direct Intramyocardial Plasmid Vascular Endothelial Growth Factor-A_{165} Gene Therapy in Patients With Stable Severe Angina Pectoris

A Randomized Double-Blind Placebo-Controlled Study: The Euroinject One Trial

Jens Kastrup, MD,* Erik Jørgensen, MD,* Andreas Rück, MD,† Kristina Tägil, MD,‡ Dietmar Glogar, MD,§ Witold Ruzyllo, MD,‖ Hans Erik Bøtker, MD,¶ Dariusz Dudek, MD,# Viktor Drvota, MD,† Birger Hesse, MD,** Leif Thuesen, MD,¶ Pontus Blomberg, PHD,† Mariann Gyöngyösi, MD,§ Christer Sylvén, MD, FACC,† the Euroinject One Group

Copenhagen and Aarhus, Denmark; Vienna, Austria; Warsaw and Krakow, Poland; and Malmö and Stockholm, Sweden

OBJECTIVES In the Euroinject One phase II randomized double-blind trial, therapeutic angiogenesis of percutaneous intramyocardial plasmid gene transfer of vascular endothelial growth factor (phVEGF-A_{165}) on myocardial perfusion, left ventricular function, and clinical symptoms was assessed.

BACKGROUND Evidence for safety and treatment efficacy have been presented in phase I therapeutic angiogenesis trials.

METHODS Eighty "no-option" patients with severe stable ischemic heart disease, Canadian Cardiovascular Society functional class 3 to 4, were assigned randomly to receive, via the NOGA-MyoStar system (Cordis Corp., Miami Lakes, Florida), either 0.5 mg of phVEGF-A_{165} (n = 40) or placebo plasmid (n = 40) in the myocardial region showing stress-induced myocardial perfusion defects on ^{99m}Tc sestamibi/tetrofosmin single-photon emission computed tomography.

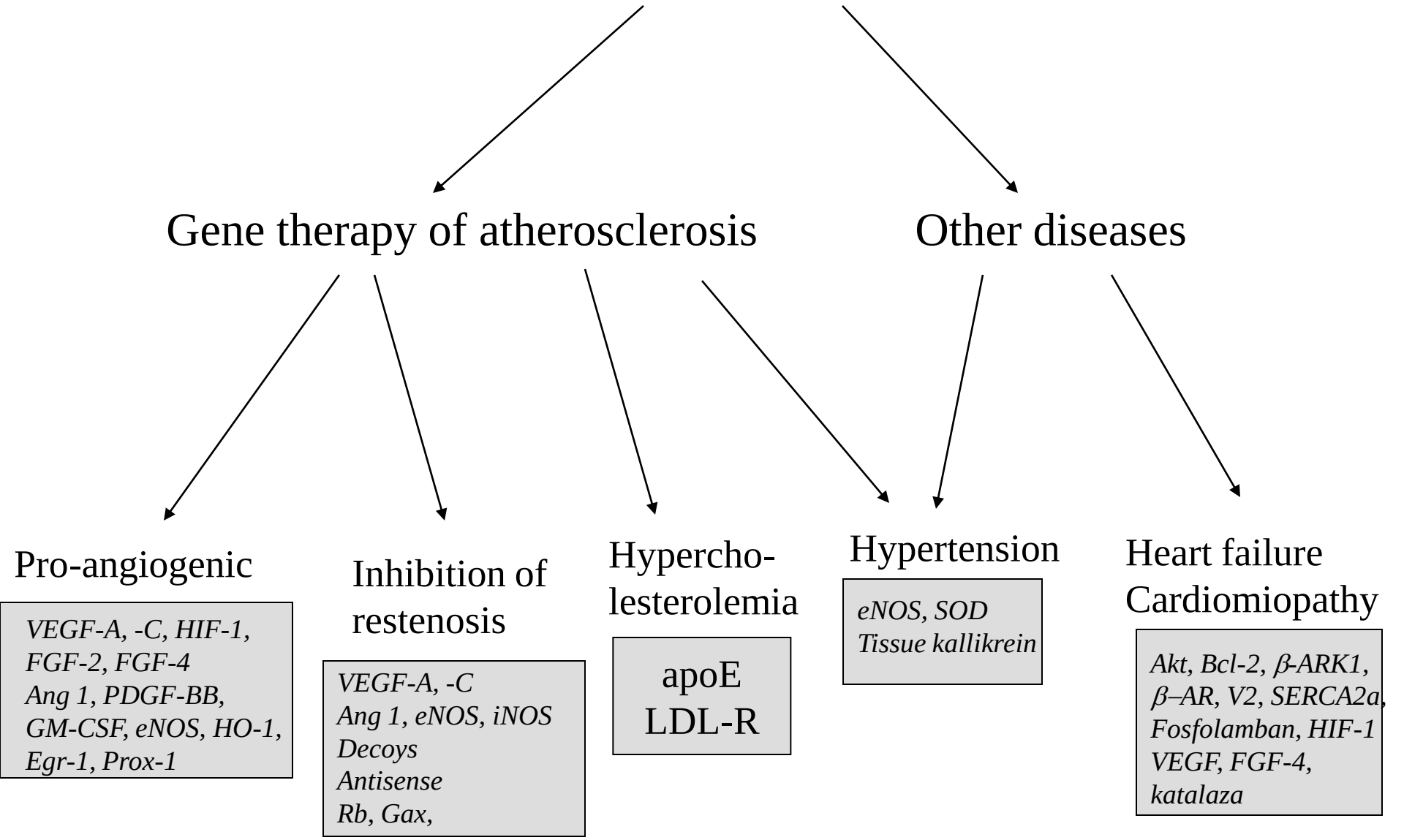
RESULTS No differences among the groups were recorded at baseline with respect to clinical, perfusion, and wall motion characteristics. After three months, myocardial stress perfusion defects did not differ significantly between the VEGF gene transfer and placebo groups (38 ± 3% and 44 ± 2%, respectively). Similarly, semiquantitative analysis of the change in perfusion in the treated region of interest did not differ significantly between the two groups. Compared with placebo, VEGF gene transfer improved the local wall motion disturbances, assessed both by NOGA ($p = 0.04$) and contrast ventriculography ($p = 0.03$). Canadian Cardiovascular Society functional class classification of angina pectoris improved significantly in both groups but without difference between the groups. No phVEGF-A_{165}-related adverse events were observed; however, NOGA procedure-related adverse events occurred in five patients.

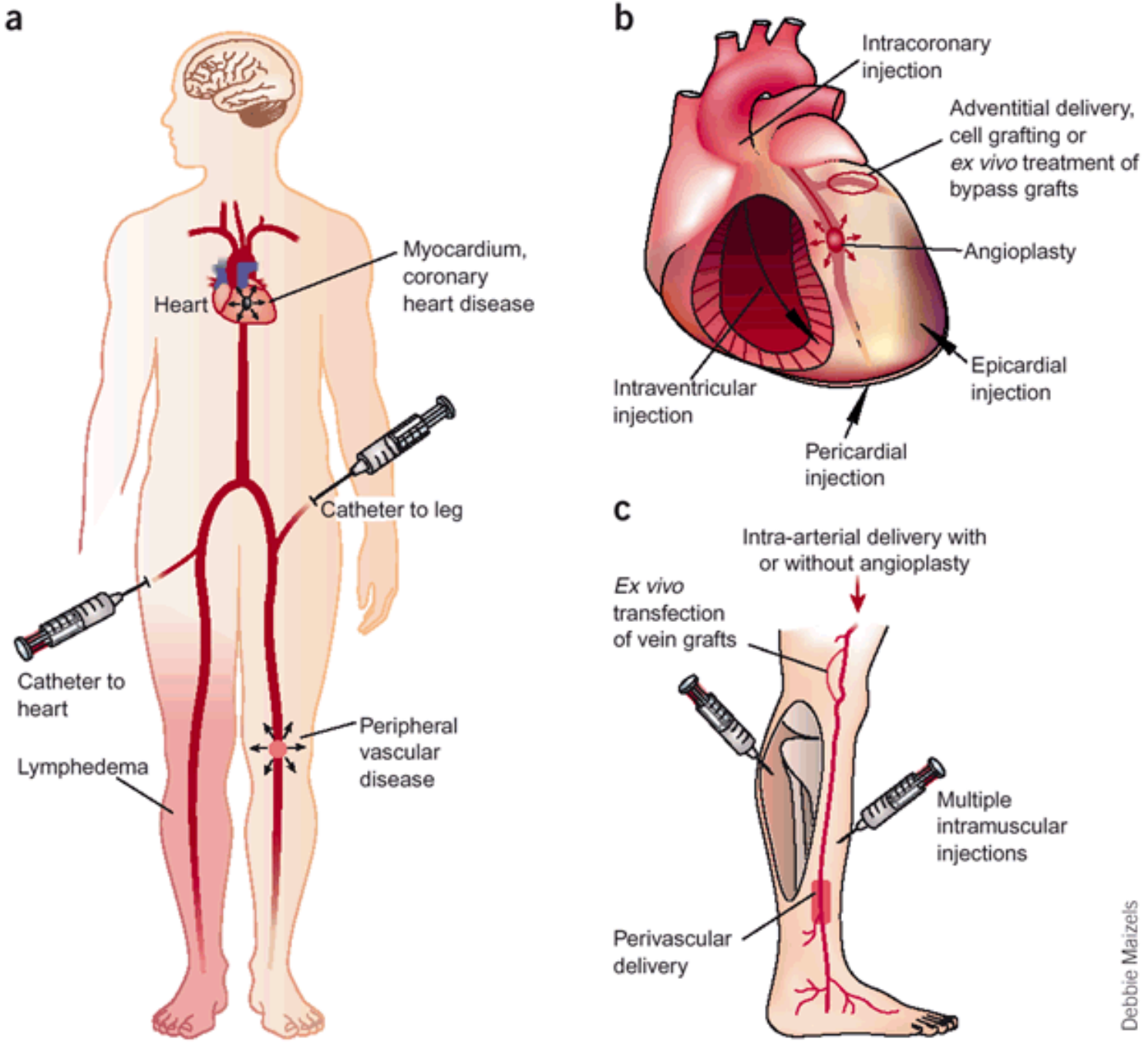
CONCLUSIONS The VEGF gene transfer did not significantly improve stress-induced myocardial perfusion abnormalities compared with placebo; however, improved regional wall motion, as assessed both by NOGA and by ventriculography, may indicate a favorable anti-ischemic effect. This result should encourage more studies within the field. Transient VEGF overexpression seems to be safe. (J Am Coll Cardiol 2005;45:982–8) © 2005 by the American College of Cardiology Foundation

VEGF increased both after plasmid and placebo...

Plasma VEGF-A, C-reactive protein, and circulating $CD34^+$ stem cells. Plasma VEGF-A increased in both groups after treatment, reaching a peak value after one week (VEGF, from 69 ± 14 ng/l to 140 ± 30 ng/l, $p < 0.001$; placebo, from 70 ± 20 ng/l to 140 ± 42 ng/l, $p < 0.001$), but without a difference between the groups. $CD34^+$ stem cells tended to be increased in the VEGF group three weeks after treatment (VEGF, from 2.8 ± 0.4 cells/10^6/l to 4.3 ± 0.6 cells/10^6/l, $p = 0.07$; placebo, from 3.2 ± 0.6 cells/10^6/l to 3.7 ± 0.4 cells/10^6/l, $p = 0.25$), with no difference between the groups. C-reactive protein increased in the VEGF gene transfer group 24 h after treatment (VEGF, from 6.4 ± 1.2 mg/l to 8.5 ± 2.0 mg/l, $p = 0.03$; placebo, from 6.2 ± 1.1 mg/l to 7.8 ± 1.9 mg/l, $p = 0.98$) with no difference between the groups.

Cardiovascular gene therapy

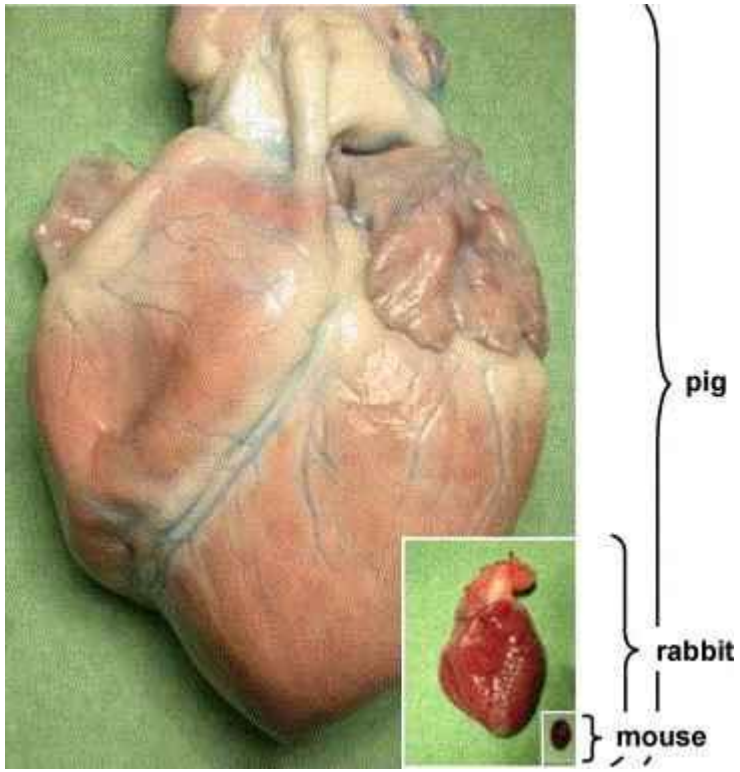


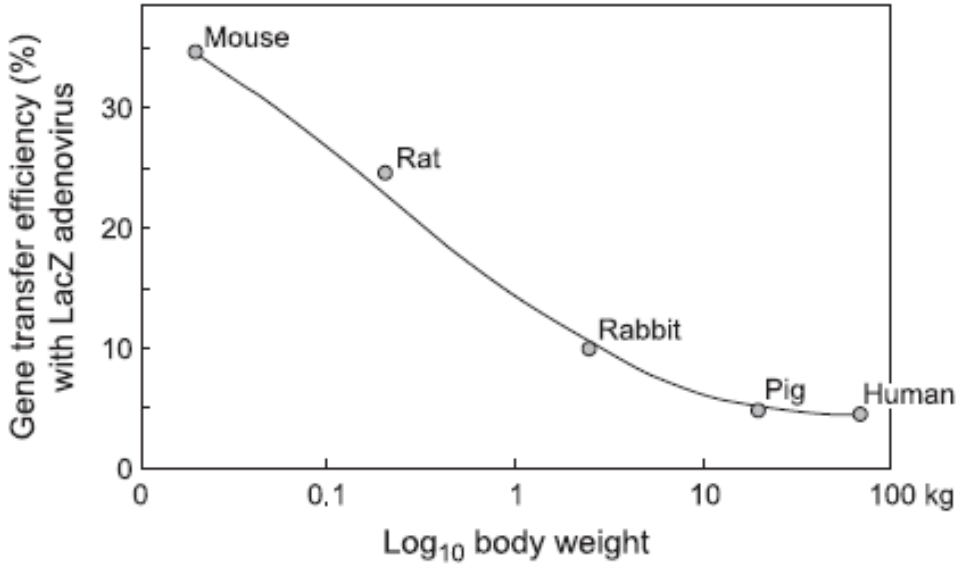


Yla-Herttuala S, Alilatalo K, Nature Med. June 2003

Why clinical trials of pro-angiogenic gene therapy are not effective?

Efficiency of gene transfer is inversely proportional to the size of animal…





Yla-Herttuala et al., Trends Cardiovasc Med., 2004

Lessons from preclinical animal models

When transducing skeletal muscle with adenoviruses expressing VEGFs, transduced cells are not only myocytes but also fibroblasts located around muscle fibres [6]. Therefore a secreted factor is needed for a therapeutic effect on muscle capillaries and other vessels. Many preclinical studies have been performed in small animal models where transduction is much more efficient than can be achieved in larger animals or human [1]. Whereas vectors may be effective in small mouse muscles, they may not effectively spread over larger muscle volumes in humans [1,17]. Also, doses used in small animals have been very high. For example, 10^{11} viral particles in a mouse would correspond to 3×10^{14} viral particles in a human [18]. A 28G needle causes a ~500 μm wide needle track in mouse muscles where damaged myocytes and inflammatory cells will become a confounding source of cytokines and growth factors. This needle track is ~20% of the total width of the left ventricle in mouse heart, but only 1/200 of the width of the human myocardium [18]. Gene therapy results from small animal models are easily overinterpreted regarding their applicability to the human clinical situation. It is obviously easier to transduce the entire muscle in the mouse than human muscle masses hundreds of times larger. Therefore further development in the transduction efficiency in humans is needed before we can expect better clinical results.

Seppo Yla-Herttuala, Biochem Transact 2009

Is there a risk of side-effects of VEGF overexpression?

Vascular endothelial growth factor

- a very potent angiogenic molecule

- specific mitogen for endothelial cells

- gene transfer of VEGF cDNA or delivery of VEGF protein has been demonstrated to be beneficial in animals studies aimed at:

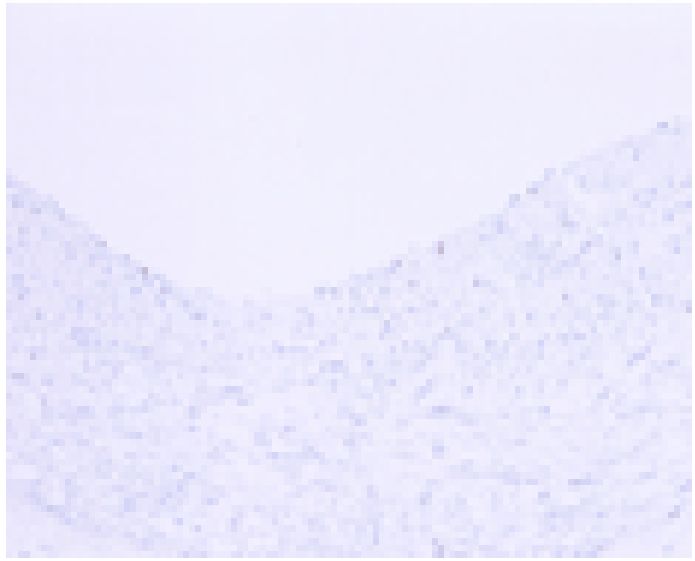
- inhibition of restenosis (secondary narrowing of the lumen of the vessel)

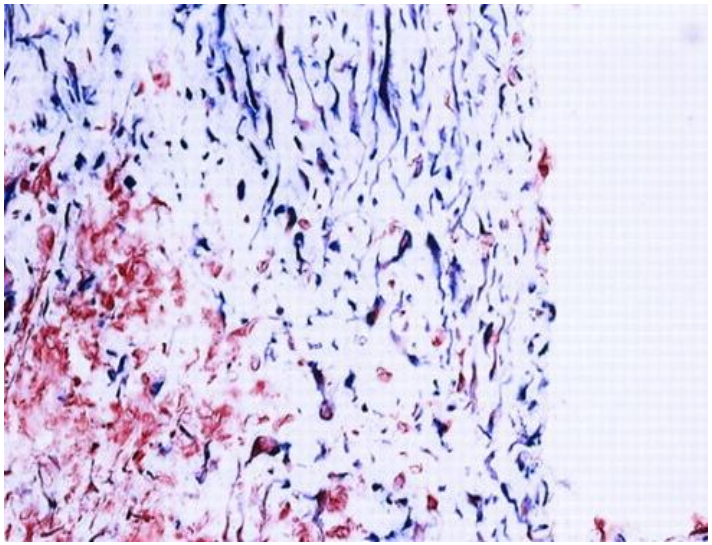
- stimulation of angiogenesis

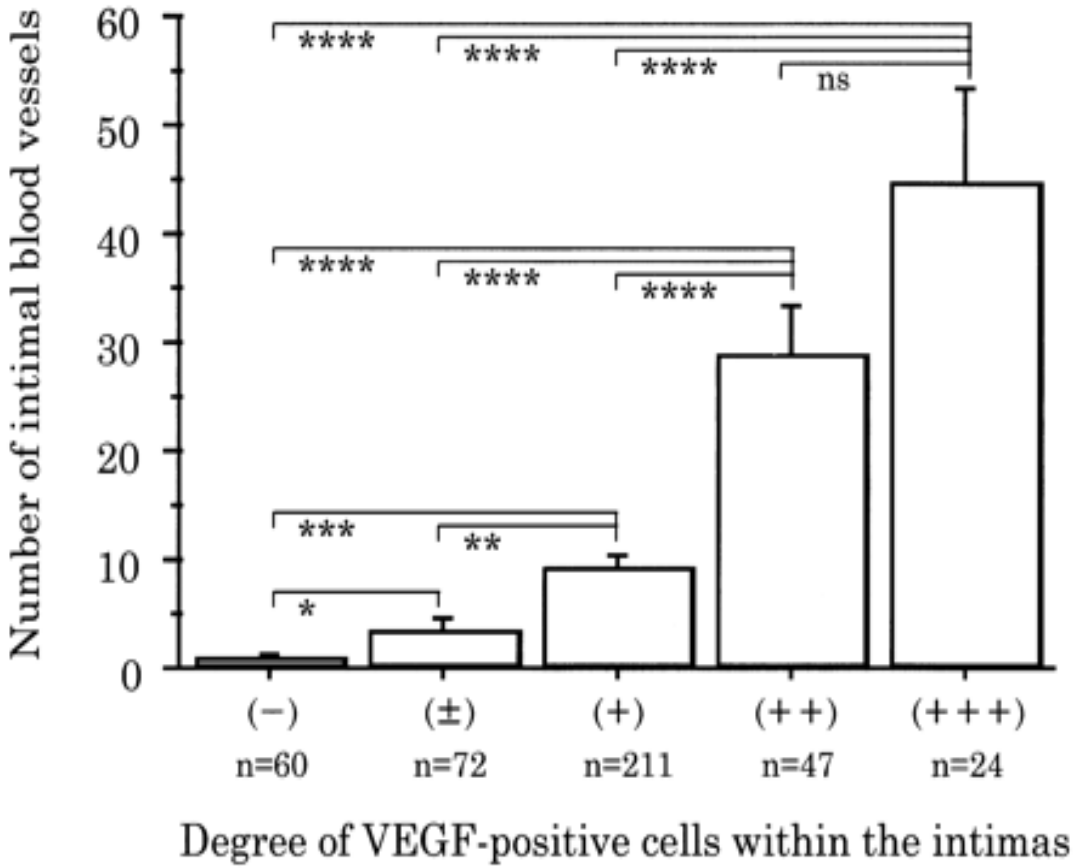
but

Is there a need to enhance VEGF production/activity in atherosclerosis?

VEGF in human atherosclerotic lesions



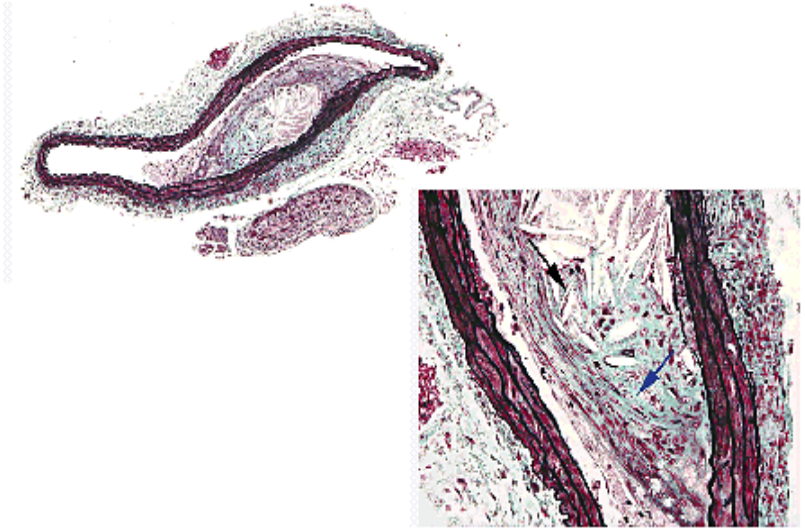




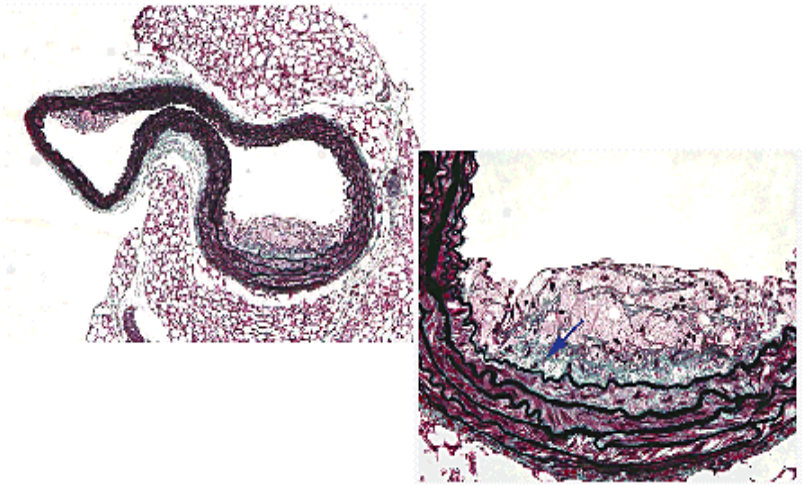
Inoue et al, Circulation, 1998

Chen et al., ATVB, 1999

VEGF enhances atherosclerotic plaque progression



Thoracic aorta of ApoE$^{-/-}$ mice treated with VEGF



Thoracic aorta of ApoE$^{-/-}$ mice treated with albumin

Celletti FA et al, Nature Med, 2001

Plaque macrphage content also increased disproportionaly over controls.
The effect dependent on VEGFR1 on macrophages (?)
Promotion of neointimal formation by acting as pro-inflammatory cytokine (?)

Conclusions (2)

1. Angiogenic gene therapy demonstrated its feasibility in numerous animal models

2. Several clinical trials have been performed in humans

3. Initially, the clinical trials demonstrated some improvements in limited number of patients

4. Large randomized trials did not show any particular long-term benefit in patients with ischemic heart disease or hind limb ischemia

5. No serious side effects have been observed in patients taking part in gene therapy trials of cardiovascular diseases; however, the potential risk of aggravations of the diseases has been suggested by several animal studies