Therapeutic Angiogenesis: The Complexities of Therapeutic Translation

ESC Basic Science Summer School, Nice 14th -18th June 2015

Professor Ian Zachary, Centre for Cardiovascular Biology and Medicine, Division of Medicine, UCL

Outline

- **Therapeutic Angiogenesis for Cardiovascular Disease: Rationale including preclinical evidence (brief overview)**
- **Clinical trials of therapeutic angiogenesis**
- **Biological and practical challenges to successful translation**
- **Lessons from clinical trials**
- **Current & alternative approaches**

Is there still unmet clinical need in Heart Disease?

- **Angina is a huge burden on health service budgets: in UK (2000) 634,000 angina sufferers cost £669m (€815m) or 1.3% of total NHS expenditure. (S. Stewart *et al;* Heart: 2003;89:848–853).**

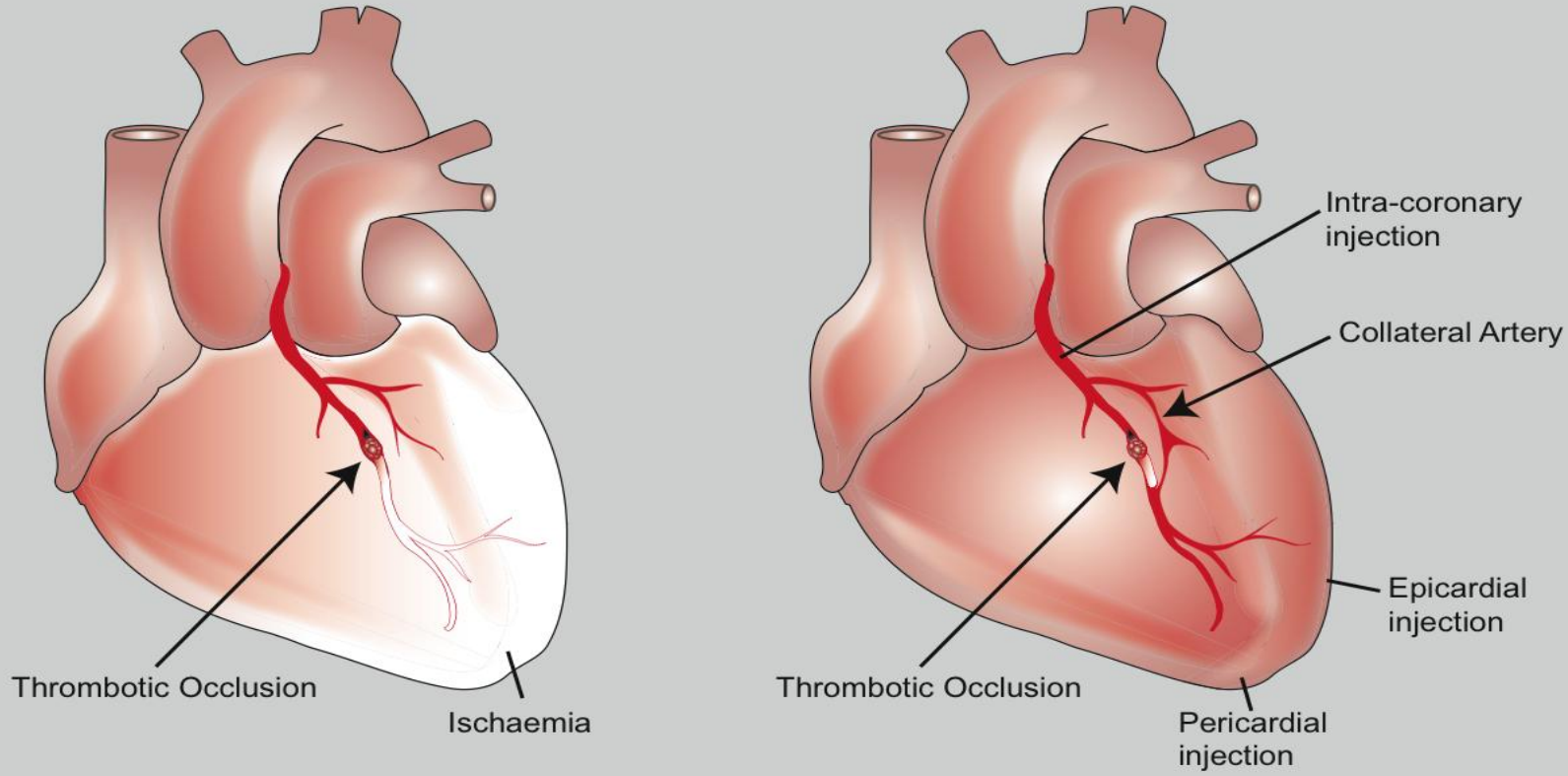
- **5-10% of patients with CHD are refractory to standard therapy. ~2.3 million US and Canadians have refractory angina (Henry T et al, Nat Rev Cardiol 2014: 11:78-95); ~100,000 new refractory angina sufferers identified in Europe each year (S. Eldabe, Trials 2013;14:57).**

- **Success in treating heart attack is creating a large group of patients with chronic heart disease leading to heart failure. There is no effective treatment for this condition.**

Therapeutic angiogenesis

- **Offers hope to “NO-OPTION” patients**
- **Angiogenic factors may stimulate collateral arteriogenesis**

Collateral arteries provide a ‘biological’ bypass for occluded atherosclerotic vessels, increasing blood flow to ischaemic heart tissue

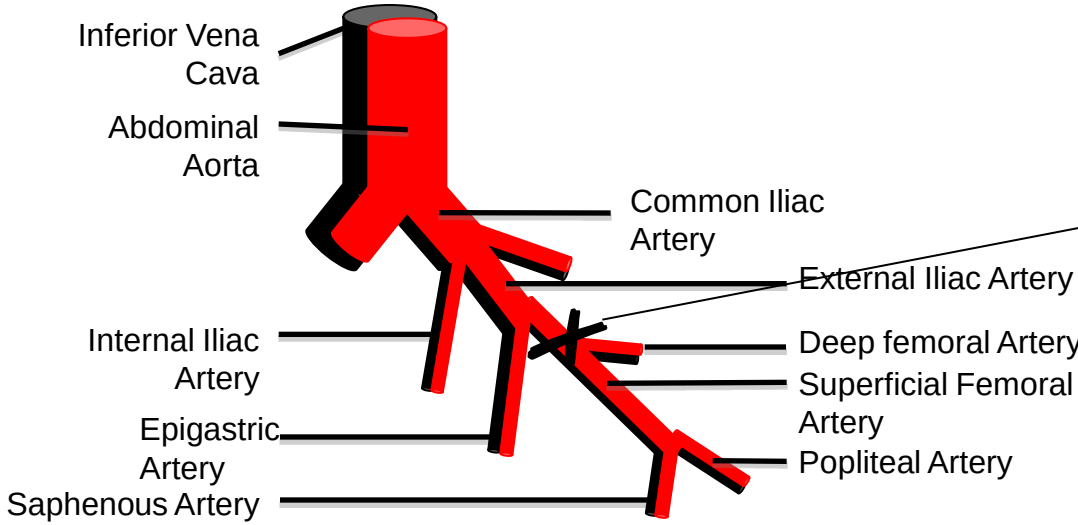


Zachary & Morgan, 2010; Heart

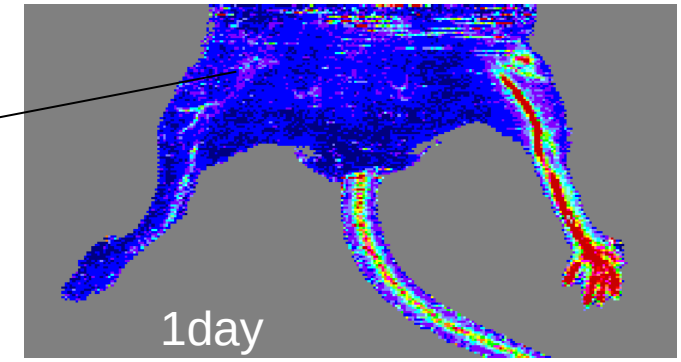
Diseases characterized or caused by insufficient angiogenesis or lymphangiogenesis

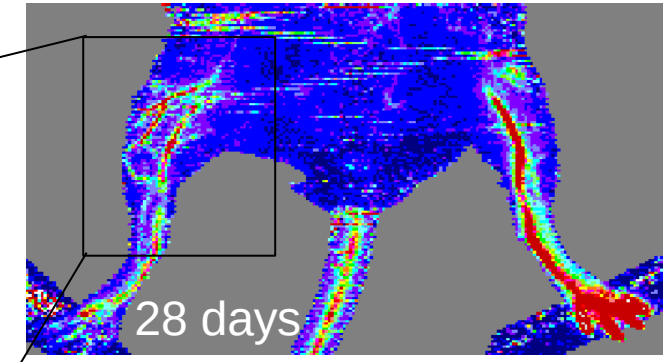
Organ	Disease
Blood and lymph vessels	Atherosclerosis, restenosis, diabetes, hypertension
Heart	**Ischaemic heart disease, cardiac failure**
Periphery (lower leg)	**Ischaemic disease**
Nervous system	Stroke; Alzheimer's disease; Diabetic neuropathy; Amyotrophic lateral sclerosis (ALS)
Gastrointestinal tract	Gastric or oral ulcerations; Crohn's disease
Skin	Hair loss; Skin purpura, telangiectasia, and venous lake formation; Systemic sclerosis, Lupus
Bone, joints	Osteoporosis, impaired bone fracture healing
Lung	Neonatal respiratory distress syndrome (RDS); Pulmonary fibrosis, emphysema
Kidney	Nephropathy (ageing; metabolic syndrome); glomerulosclerosis; tubulointerstial fibrosis
Reproductive system	Preeclampsia; Intrauterine growth retardation; Menorrhagia (uterine bleeding)

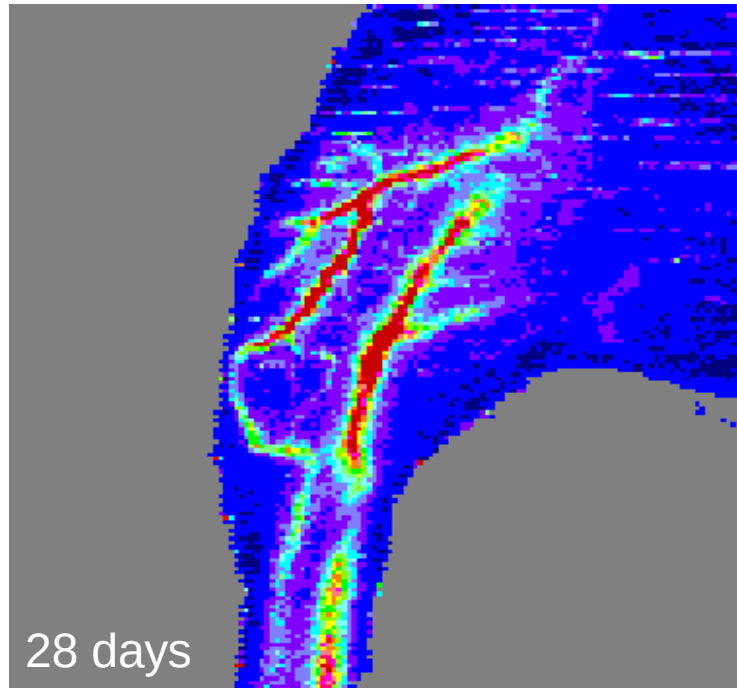
Revascularisation in the Mouse Hindlimb ischemia model



Scanning laser-doppler imaging

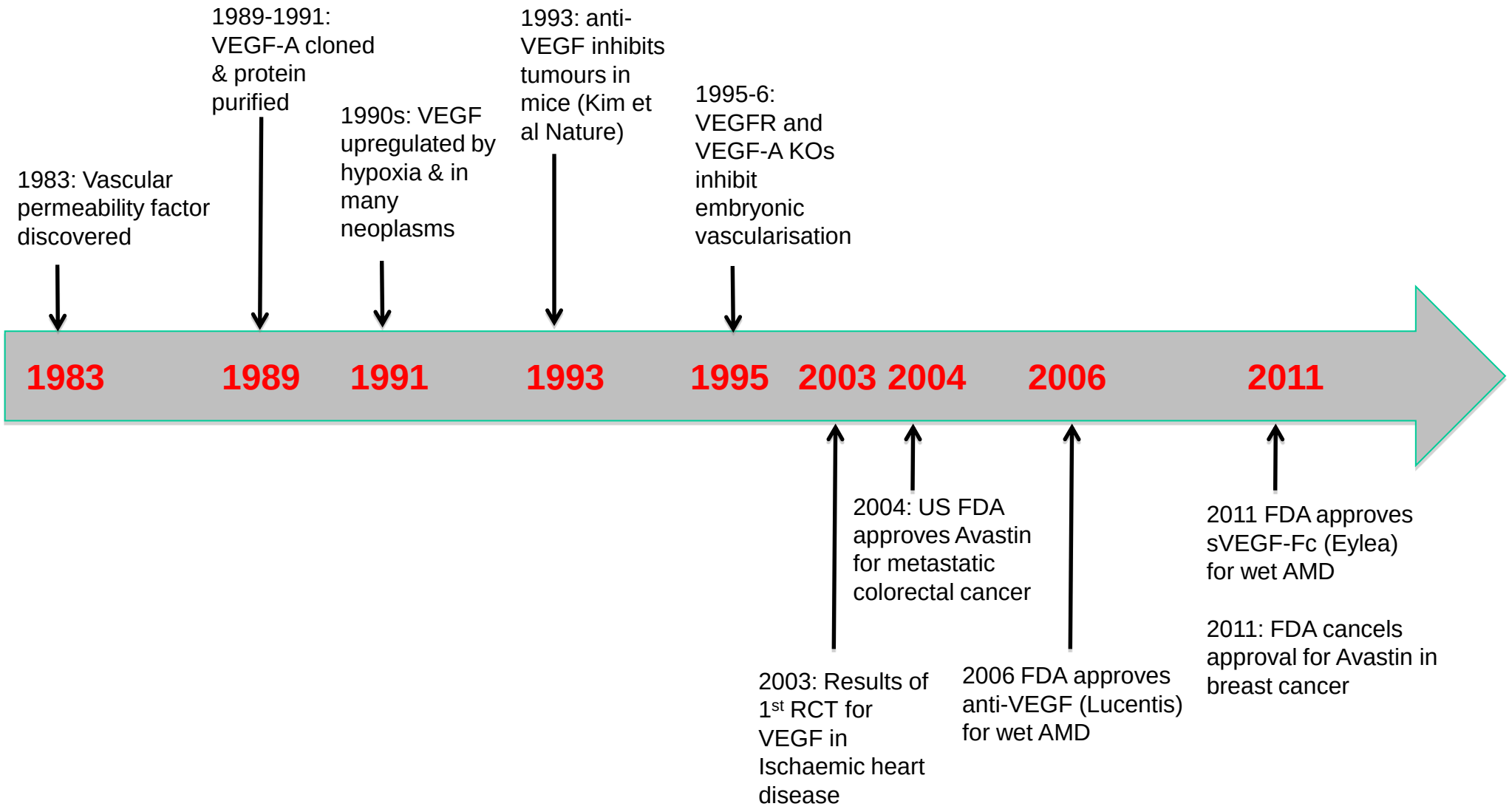






Thanks to Dr Caroline Pellet-Many

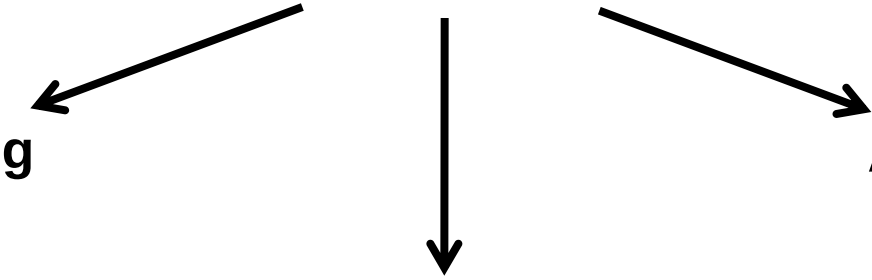
Vascular Endothelial Growth Factor (VEGF-A): Translation from bench to bedside



Therapeutic potential of VEGF-A for Cardiovascular Disease

VEGF-A

- Increases endothelial survival, migration, proliferation & production of arterioprotective factors (NO, prostacyclin) - Regenerative and protective
- $VEGF_{165}$ is secreted and diffusible – 'by-stander effect'
- Essential for angiogenesis in development and in disease, important for maintenance of adult vascular health



Angioplasty,stenting or CABG: **Accelerated Re-endothelialsation**

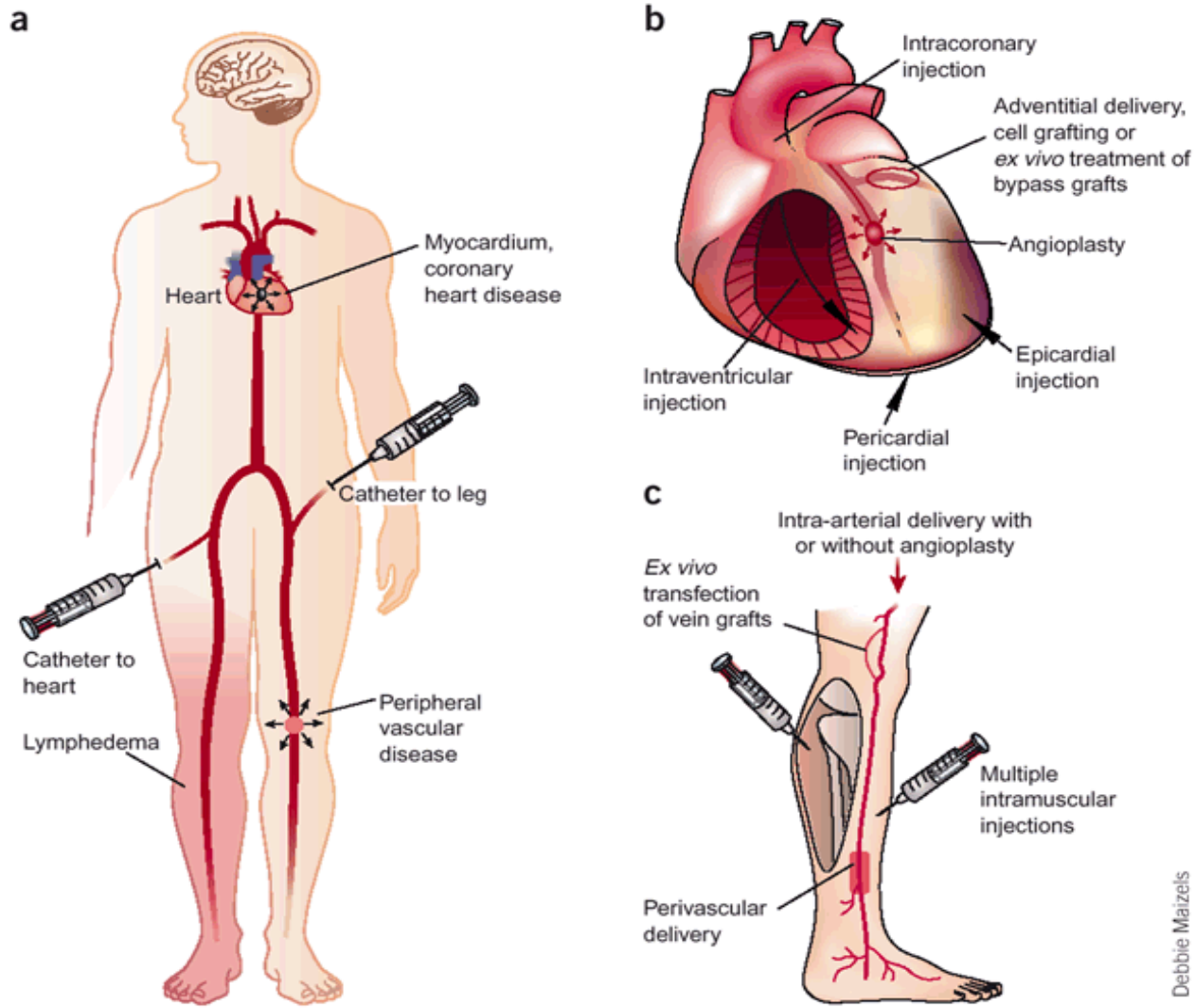
Cardiac & peripheral ischaemic diease: **Therapeutic angiogenesis**

Angioplasty,stenting or CABG: **Vascular protection**

Studies of VEGF delivery in animal models of vascular insufficiency and neointima formation

Model	Species	VEGF	Result
Limb ischemia	Rabbit hindlimb	Protein intravenous	Increased revascularization[13]
			Recovery of collateral endothelium-dependent flow[16]
			Evidence of enhanced collateral formation[114]
			Increased cell proliferation and collateral formation[115]
		Protein intramuscular	Increased limb perfusion and increased collateral formation[116]
			Improved muscular blood supply[18]
			Increased collateral supply[117]
		cDNA intramuscular	Improved collateral blood supply and increased capillary density[118]
	Rat hindlimb	cDNA intramuscular	Restoration of vasomotor responses[119]
	Canine hindlimb	Protein intravenous	Improved collateral circulation[23]
Myocardial ischemia	Porcine	Protein intravenous	Increased collateral-dependent flow and hypotension[20]
			Increased coronary flow[19]
			Increased collateral blood supply shown by MRI[15]
		Protein intracoronary	Increased angiogenesis[21]
		cDNA ($VEGF_{121}$)	Collateral formation and increased perfusion[22]
	Canine	Protein intravenous	Increased angiogenesis and collateral flow[14]
			No increase in collateral formation and increased neointimal hyperplasia[37]
Balloon injury	Rat carotid	Protein intravenous	Accelerated reendothelialization[9]
		cDNA intravenous	Accelerated reendothelialization[10]
Stent implantation	Rabbit iliac	Protein intravenous	Accelerated reendothelialization, decreased neointima formation and reduced mural thrombosis[11]
		cDNA intravenous	Accelerated reendothelialization, decreased neointima formation and reduced mural thrombosis[12]
Vein graft	Rabbit	Protein topical	Decreased neointima formation[24]
Extravascular silastic collar	Rabbit carotid	cDNA local extravascular	Reduced neointima formation in presence of intact endothelium and absence of angiogenesis[55]

Gene Delivery to the Cardiovascular System



Yla-Herttuala S. & Alitalo K. *Nature Med*. 2003; 9: 694-701

The rationale for Clinical Trials of Therapeutic Angiogenesis

- **Collateral vessel formation occurs naturally**
- **Several therapeutic candidates (eg VEGF, FGF-2)**
- **Strong pre-clinical evidence from several animal species & models of ischaemic disease supports therapeutic benefit of angiogenic cytokines**
- **Feasible to deliver cytokines to the cardiovascular system via multiple routes**
- **Suitable patient groups for whom standard revascularisation is not an option**

Phase 1/2 placebo-controlled, double-blind , dose-escalating trial of catheter-mediated VEGF gene transfer for chronic myocardial ischaemia

Gene therapy protocol

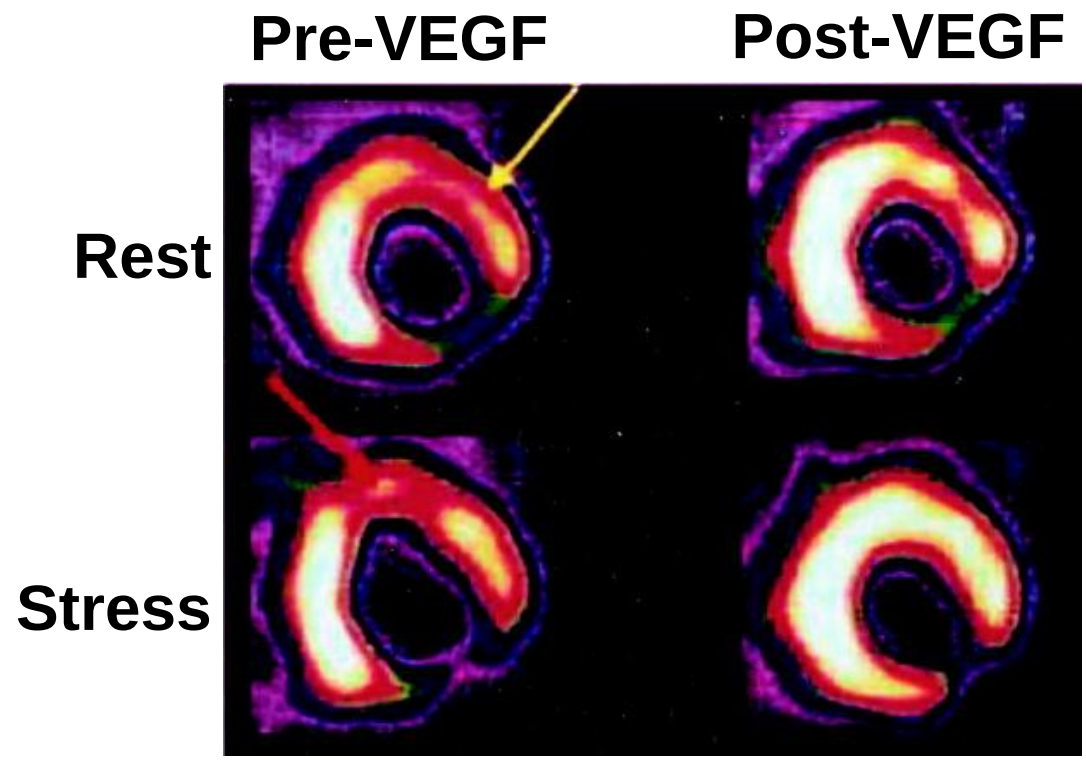
19 patients with class III/IV angina not suitable for conventional revascularisation

Naked DNA delivered to left ventricular myocardium:
12 received VEGF-2
7 received placebo

Results

At 12 weeks significant improvement in angina class and trend to improvement in exercise tolerance
Evidence of improved perfusion from SPECT in VEGF-2 versus placebo

SPECT myocardial perfusion before and after VEGF gene therapy



Losordo et al *Circulation* 2002; 105: 2012-2018

VIVA Trial (Genentech Inc)
VEGF in Ischaemia for Vascular Angiogenesis

Double-blind, placebo-controlled, randomised trial in 178 patients with refractory angina to placebo or one of two doses (17ng/kg/minute or 50ng/kg/minute) of IC and IV rVEGF.

Patients with viable underperfused myocardium who are not optimal candidates for CABG or PTCA

Placebo n=63

17 ng/kg/min n=56

50 ng/kg/min n=59

rhVEGF$_{165}$: IC + IV x 3

Efficacy endpoints (60 and 120 days)

Treadmill time
Clinical
SPECT - myocardial perfusion
Angiography

Henry et al. Circulation.2003;107:1359-65.

VIVA Trial: conclusions

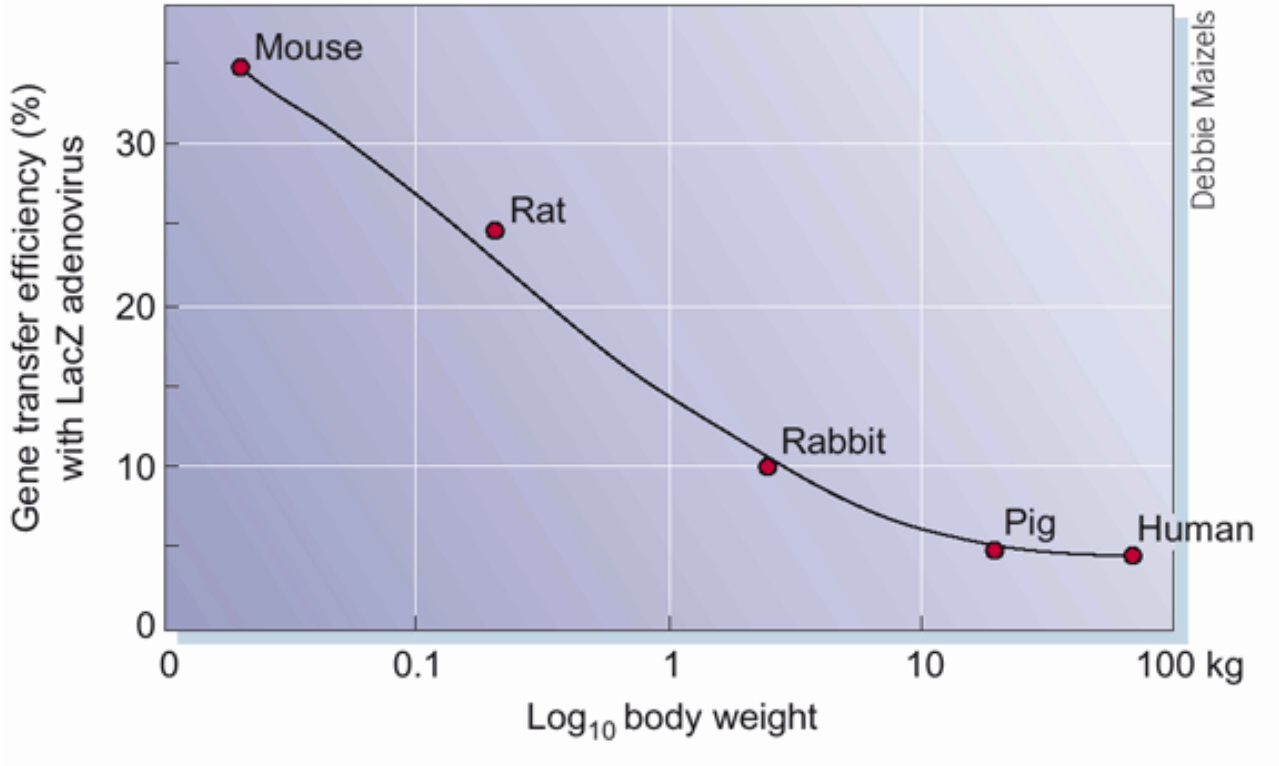
- **Prominent placebo effect at 60 days**
- **No significant differences in treadmill times, nuclear perfusion or angiography at 60 days**
- **Trend to improvement in angina class and frequency and treadmill times at 120 days**
- **Pharmacokinetics show that blood VEGF levels return to basal after 2-8 hours**
- **Safe - no increase in malignancies after 1-2 year follow-up**

Other clinical trials of angiogenic protein, plasmid and adenoviral gene therapies have not produced significant patient benefit

Why have Therapeutic Angiogenesis Trials been so Disappointing?

- **Treatments do not stimulate sufficient angiogenesis or collateral vessel formation –**
  - → **Dosing, route(s) & site of administration suboptimal**
  - → **Proteins have short half-life, and dose/frequency limited by acute vascular effects**
  - → **Plasmids do not transfect target tissue efficiently**
  - → **Even adenoviruses may only transduce 5-10% of target cell population**
- **Angiogenesis is stimulated, but vessels are not viable or appropriate for revascularisation**
- **Is angiogenesis beneficial or harmful for cardiovascular disease?**

Size matters: Gene transfer efficiency inversely related to size of organism

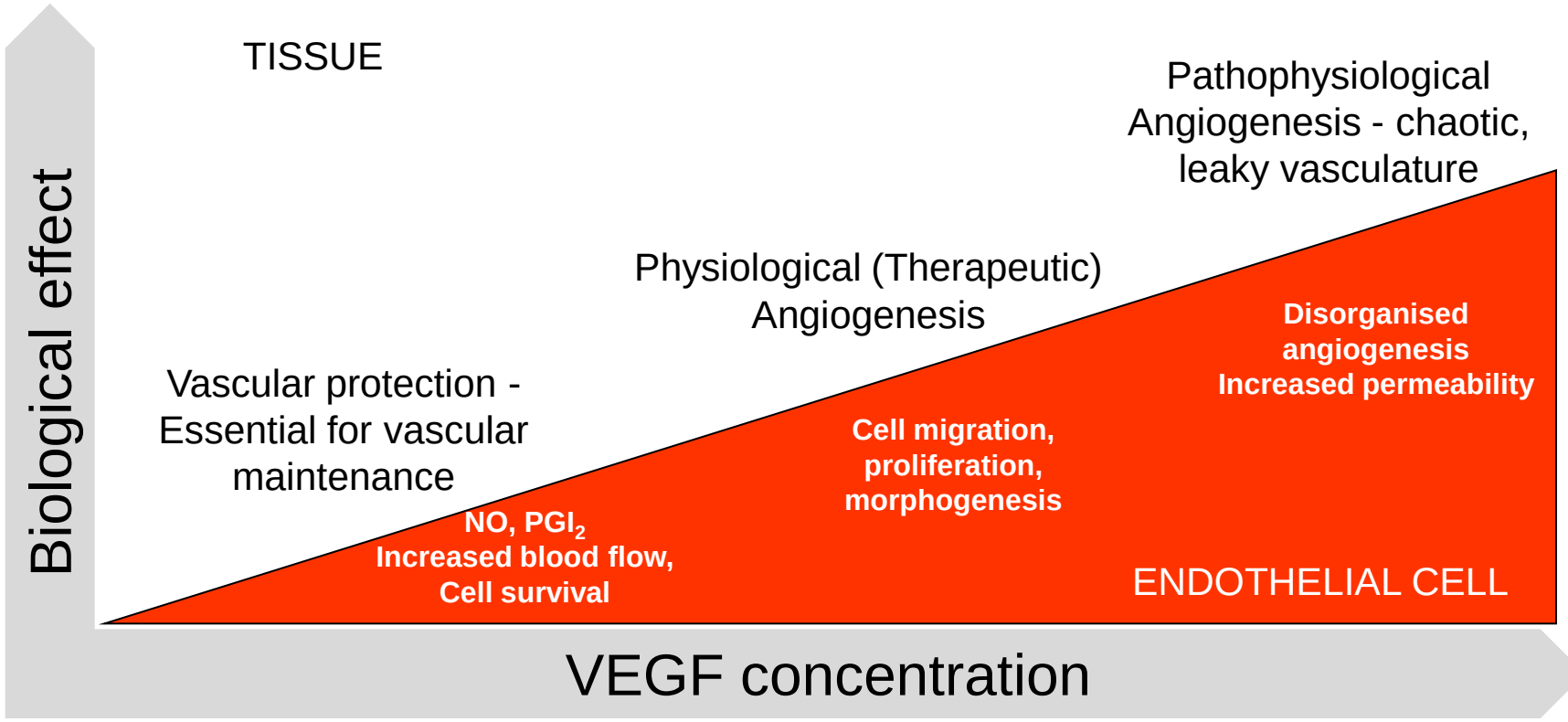


Ad.LacZ in sheep artery

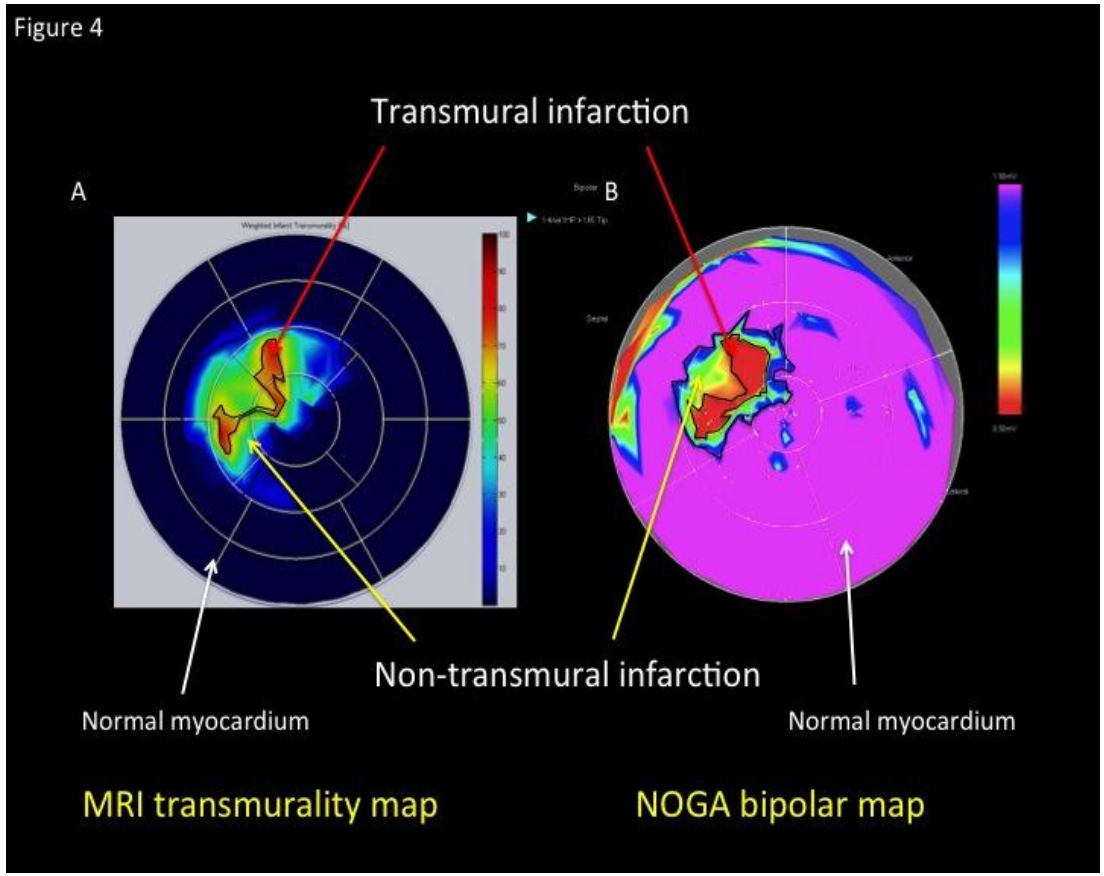
Dr Vedanta Mehta

Yla-Herttuala S. & Alitalo K. *Nature Med*. 2003; 9: 694-701

Dose matters: Concentration-dependent Effect of VEGF in the Cardiovascular System



Targeting Angiogenic Gene Therapy: NOGA-guided delivery



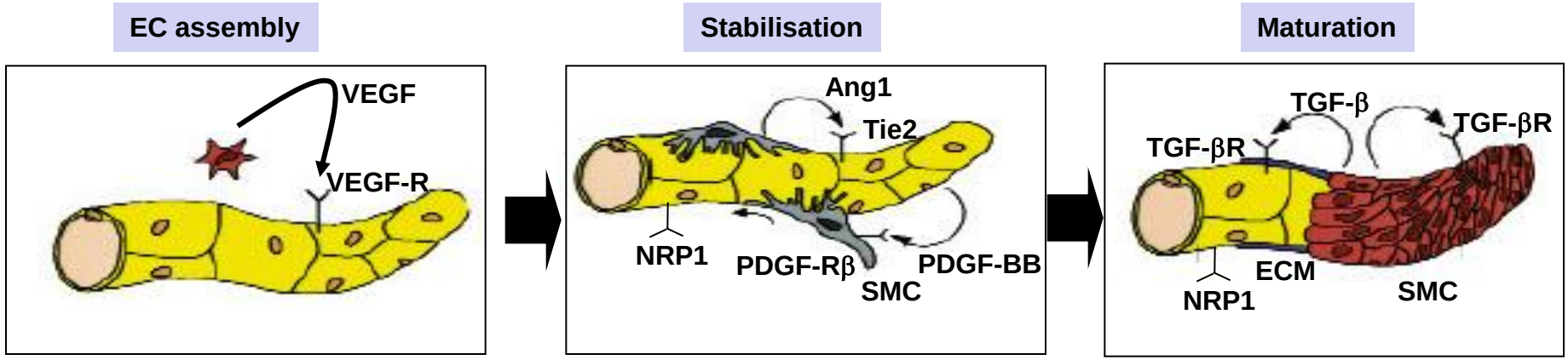
- The border zone of myocardial infarction (MI) have decreased viability and reduced wall motion capacity (hibernating myocardium). NOGA recognizes preserved electrical activity indicating hibernating myocardium. Regenerative therapies delivered to these areas may better restore cardiac function.
- NOGA endocardial mapping correlates well with Cardiac magnetic resonance imaging (cMRI) with gadolinium enhancement (LE), but cMRI is an off-line imaging modality, and there is a delay between diagnostic imaging and application of the therapy when the patient is in the cath lab.
- The NOGA only technique for nonfluoroscopic mapping of the heart and 3D navigation during percutaneous intramyocardial delivery of regenerative therapies.

Pavo et al *PLoS One*. 2014 (11):e113245

Therapeutic angiogenesis - biological complexities

- **Healthy animal models of ischaemic myocardium & periphery do not model human disease: age, pathology, medication, co-morbidity**
- **Are monotherapies (eg VEGF) sufficient to stimulate a therapeutically beneficial biological response in the adult heart?**
- **Do VEGF and angiogenesis have harmful effects in CHD patients?**

Role of Pericytes and Vascular Smooth Muscle Cells (VSMC) in Vessel Maturation

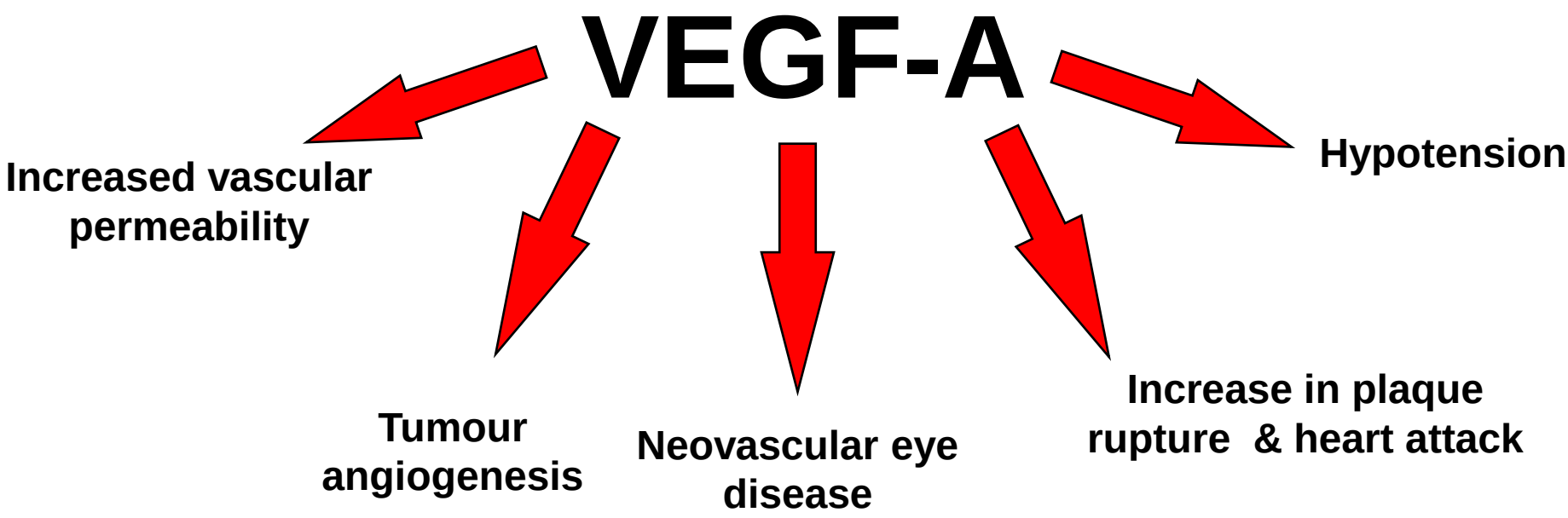


Carmeliet P. *Nature Med*. 2000; 6: 389-395

Is Combination Therapeutic Angiogenesis a Solution?

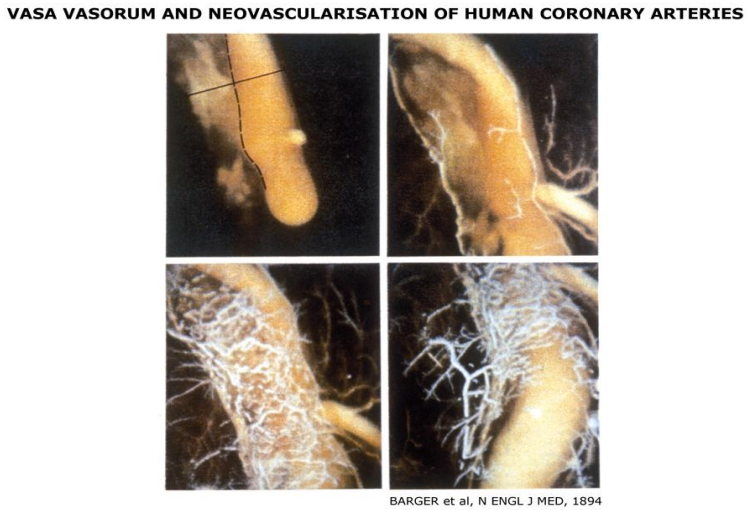
- **Limited pre-clinical evidence**
- **Difficult to conduct combination therapy trials with separate proteins or vectors**
- **VEGF-A165 plus bFGF given by intramyocardial injection in CHD patients found no change in myocardial perfusion, although exercise capacity and symptoms improved. (Kukula et al. 2011). The combination of G-CSF mobilization of bone marrow stem cells and plasmid VEGF-A165 was without any improvement in symptoms or myocardial perfusion (Ripa 2006).**

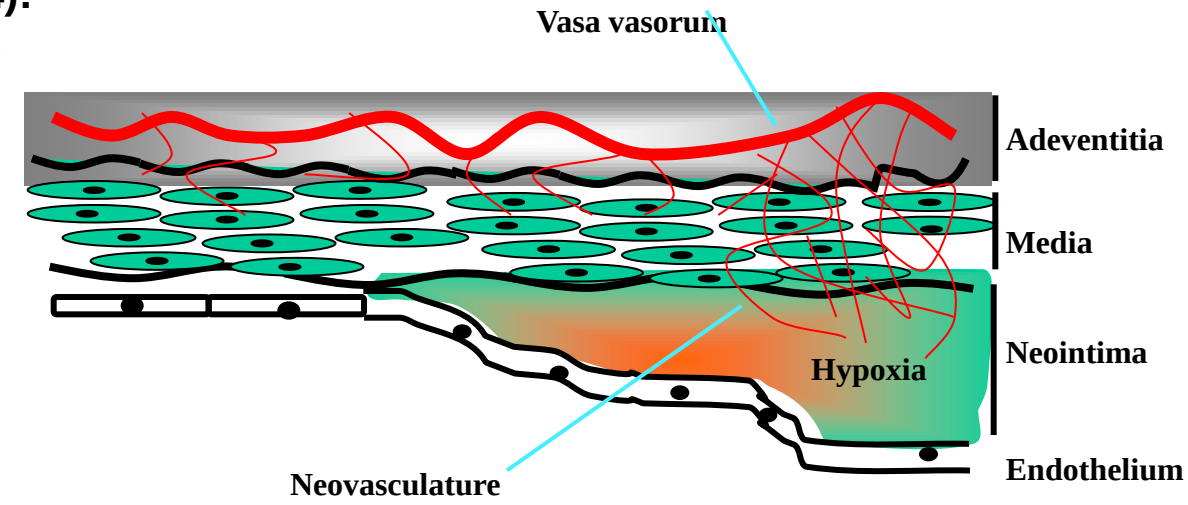
Potential harmful effects of VEGF-A therapy



Does Angiogenesis promote Atherosclerosis?

Hypothesis (Barger et al *N Eng J Med* 1984):





- Adventitial neovascularisation allows coronary atherosclerotic plaques to develop beyond a critical thickness by supplying oxygen and nutrients to the core of the lesions.

-The neovasculature in coronary atherosclerotic plaques is more fragile and prone to rupture, and a potential cause of plaque destabilization, leading to acute coronary syndromes

Angiogenesis inhibitors reduce, and VEGF enhances atherosclerotic plaque progression in apolipoprotein E-deficient mice (Moulton et al. *Circulation*. 1999;99:1726-32; Celletti et al *Nat Med*. 2001;7:425-9).

VEGF and other markers of angiogenesis, hypoxia and inflammation are expressed in human atherosclerotic plaques

Microvessels (capillaries) are a common feature of advanced human coronary atherosclerotic plaques, and often found in most vulnerable regions of plaques, and associated with the severity of disease.

Evidence against a Pro-atherogenic role of VEGF from Animal Models & Human studies

- **No evidence of plaque destabilization or rupture in animal models. Intra-plaque microvessels are rare in murine, rabbit and pig models of atherosclerosis.**

- **VEGF-A protein and Adenoviral gene transfers of VEGF-A, VEGF-B, VEGF-C and VEGF-D have no effects on atherosclerosis in hypercholesterolemic LDL-receptor/ApoB48-deficient mice (Leppänen et al. *Circulation* 2005; 112:1347-52).**

  - High circulating level of VEGF

  - Cholesterol mainly in LDL - similar to human disease

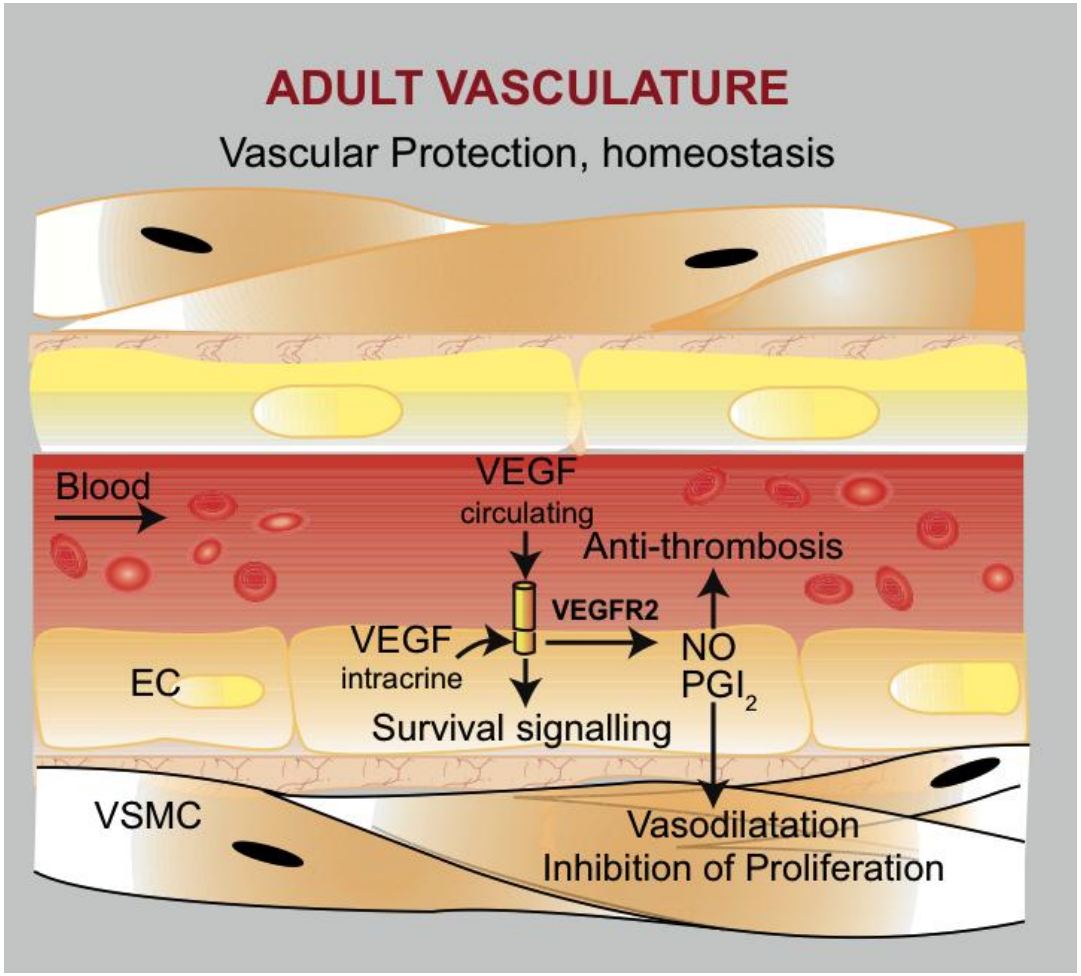
- **Inhibition of VEGFR2 using anti-Flk antibody has no effect on plaque size or vessels in ApoE-deficient mice (Luttun et al. *Nat Med*. 2002;8:831-40)**

- **Clinical trials of VEGF and other angiogenic cytokines in >2,500 patients with ischaemic heart disease reveal no evidence of increased incidence of cardiovascular disease, cancer, or diabetes. Confirmed in long-term follow-up.**

- **Clinical trials of VEGF inhibitors in cancer show increased incidence of cardiovascular side-effects, consistent with an protective role of VEGF in the adult vasculature**

See Khurana et al Circulation 2005; 112: 1813-1824 for references

Role of VEGF in vascular homeostasis & maintenance of healthy endothelial function in vivo



- **VEGF stimulates endothelial production of NO and prostacyclin**
- **VEGF increases blood flow and reduces blood pressure in animal models of peripheral and cardiac ischaemic disease**
- **VEGF required for maintenance of microvasculature & adult function in some tissues and organs (eg Kidney).**
- **VEGF-targeted anti-angiogenic drugs have cardiovascular toxicity, eg hypertension (increased blood pressure).**

Therapeutic angiogenesis: Conclusions & Perspectives

- **Difficult to translate pre-clinical studies into proven benefit for patients. Larger trials needed.**
- **VEGF (gene and protein) treatment for ischaemic heart disease is safe and well-tolerated**
- **Some trials ongoing, eg AWARE, KAT301'Endocardial VEGF-D Gene Therapy for Severe Coronary Heart Disease' (see https://clinicaltrials.gov).**
- **Combination therapies (eg VEGF + PDGF or angiopoietin or FGFs), or delivery via stem cells may be alternative approaches**

Thank you - Questions

Staff, collaborators, Funders

Centre for Cardiovascular Biology & Medicine, UCL

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British Heart Foundation

SEVENTH FRAMEWORK PROGRAMME

BBSRC bioscience for the future

KAT301'Endocardial VEGF-D Gene Therapy for Severe Coronary Heart Disease' (see https://clinicaltrials.gov)

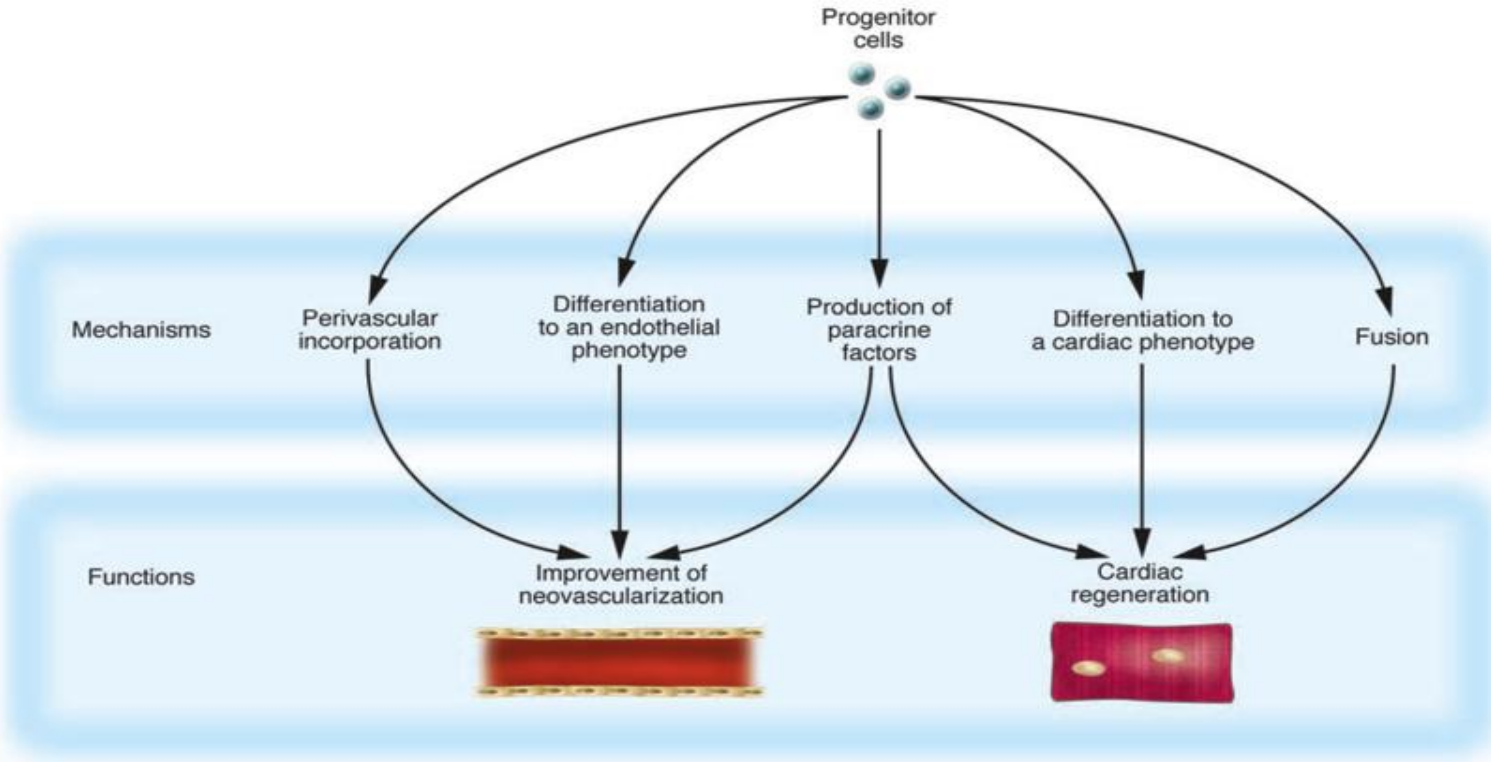
Seppo Yla-Herttuala, University of Eastern Finland, Kuopio

- Preclinical evaluation of AdVEGF-D$^{\Delta N \Delta C}$ in the pig model **of acute pig myocardial infarction** (Lähteenvuo *et al*, 2013).
- AdVEGF-D$^{\Delta N \Delta C}$ stimulates angiogenesis in vivo but has improved safety profile compared with AdVEGF-A: reduced effects on permeability & inflammatory cell infiltration
- Randomized, controlled, double-blinded, multicentre phase I/II study of the efficacy, proof-of-concept and safety of NOGA catheter-based targeted endocardial delivery of adenovirus encoding vascular endothelial growth factor-D (AdVEGF-D$^{\Delta N \Delta C}$) in 150 'no-option' patients with severe CHD for whom revascularisation cannot be performed.
- Primary endpoint: improvement in exercise capacity and relief of angina pectoris symptoms 6 months after the treatment; secondary endpoints: safety (major cardiac adverse events, MACE), quality of life, surrogate imaging assessing myocardial function, perfusion and angiogenesis using SPECT, PET, MRI and ultrasound.

KAT301‘Endocardial VEGF-D Gene Therapy Trial: Update

- 30 patients recruited.
- dose selected based on the dose escalation part of the study is 10^{-11} viral particles of VEGF-ΔNΔC
- Safety appears excellent based on 3 months protocol-defined midterm safety review
- Results may be ready for ESC/AHA 2015

‘Stem Cell’ or Endothelial Progenitor Cell Therapy: Mechanisms of action



Studies in animal models suggest that bone marrow-derived or circulating endothelial progenitor cells (EPC) promote endothelial regeneration in arterial injury and collateral angiogenesis in the ischaemic heart

Dimmeler S, Zeiher AM, Schneider MD *J Clin Invest* 2005:115;572.

Results of Stem/Progenitor Cell Trials

- **Systematic meta-analysis of the effect of bone marrow derived stem cell therapy following acute MI in trials involving 811 patients concluded that left ventricular function improved by 2.99% and lesion size decreased by 3.51% compared with controls.**
- **Whether such relatively modest effects could be clinically beneficial in terms of mortality or quality of life is at present unclear.**

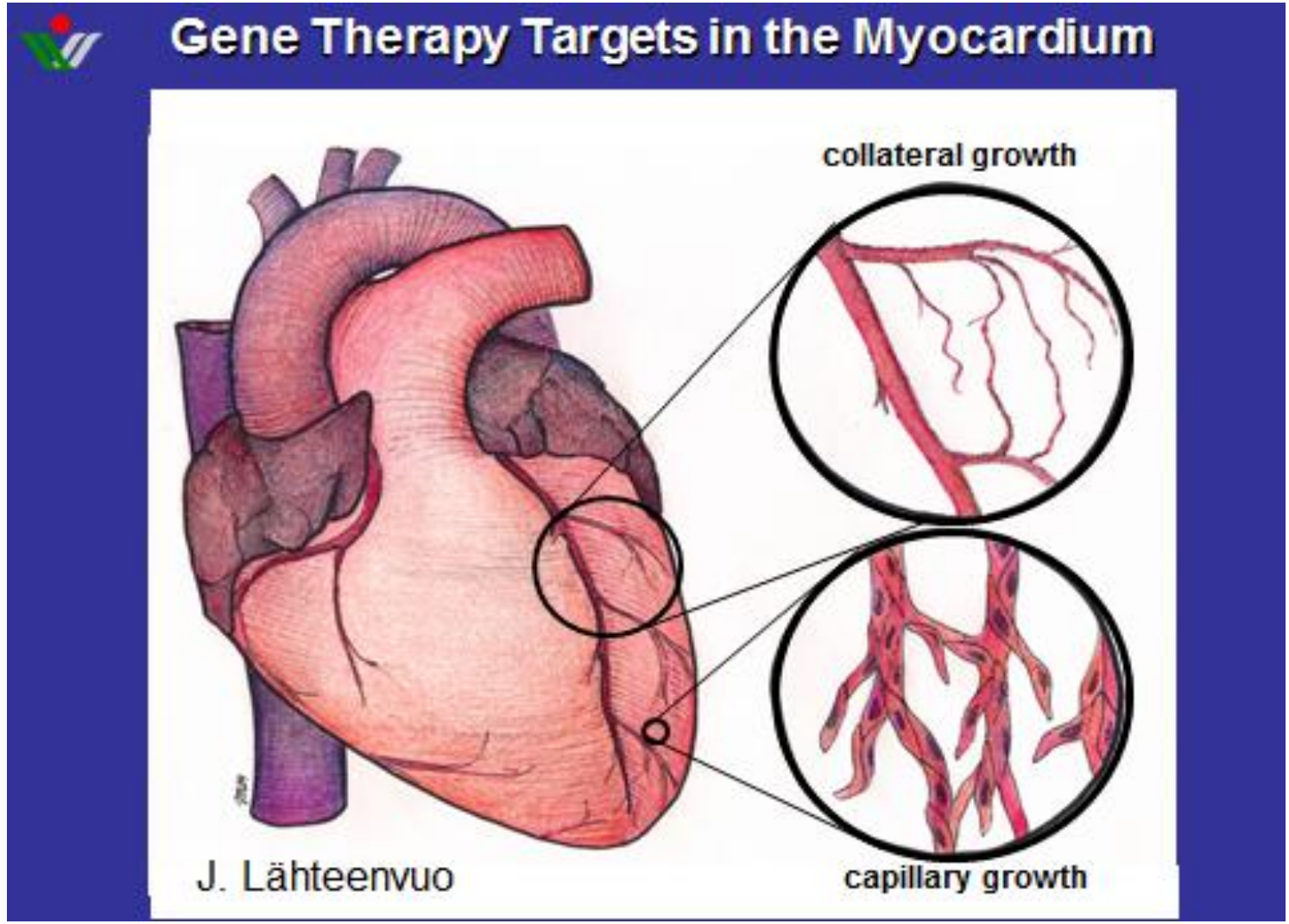
Factors with potential in cardiovascular therapeutic angiogenesis therapy

Therapeutic application	Desired biological effect	Therapeutic factor	Target for Inhibition
Promotion of growth of blood vessels and lymphatic vessels	Stimulation of capillary growth (angiogenesis), collateral artery growth (arteriogenesis) and lymphatic vessel growth (lymphangiogenesis)	VEGF (VEGF-A), PlGF, VEGF-B, -C, -D and –E, EG-VEGF, FGF-1, -2, -4 and -5, Ang-1 and -2, HGF, PDGF-A, -B, C- and –D, IGF-1 and -2, HIF-1α, MCP-1, GM-CSF, eNOS, kallikrein, EGR-1, Ets-1, Del-1, PR39, Id1, stromal cell-derived factor-1, platelet-derived endothelial cell growth factor/thymidine phosphorylase, adrenomedullin, sonic hedgehog, secretoneurin, thrombopoietin, netrin-1 and -4	Ezrin
Vascular protection, and prevention of restenosis, in-stent restenosis, and graft failure	Enhanced endothelial function (vasodilatation and anti-thrombosis), endothelial regrowth/repair, reduced SMC proliferation and migration, anti-inflammation, inhibition of excess matrix production, induction of apoptosis	VEGFs, eNOS, iNOS, prostacyclin, ecSOD, hemeoxygenase-1, catalase, TIMPs, HGF, p53, p21, p27-p16 Chimera, RB2/p130, Ras, Fas ligand, thymidine kinase, β-interferon, lipoprotein-associated phospholipase A2, C-type natriuretic peptide, PPARγ, Forkhead, β-adrenergic receptor kinase, CGRP, RAD50, TGF-3, soluble TGF-β type II receptor, kallikrein, homeobox gene Gax	PDGF-B, PDGFR-β, FGF-2, E2F, COX, ICAM, VCAM, midkine, activator protein-1, PAI-1, Rho kinase, G, CDC2 kinase, cyclin B1, cyclin G1, MCP-1, TNFα

Abbreviations: Ang, angiopoietin; β-AR, β-adrenergic receptor; COX, cyclo-oxygenase; ecSOD, extracellular superoxide dismutase; EGR-1, early growth response factor-1; EG-VEGF, endocrine gland-derived VEGF; eNOS, endothelial nitric oxide synthase; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; ICAM, intercellular adhesion molecule; IGF, insulin-like growth factor; MCP-1, monocyte chemoattractant protein 1; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; PlGF, placenta growth factor; PPARγ, peroxisome proliferator-activated receptor; SEK-1, stress-signaling kinase; SMC, smooth muscle cell; TGF, transforming growth factor; TIMP-1, tissue inhibitor of metalloproteinase 1; TNFα, tumor necrosis factor; tpa, tissue plasminogen activator; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

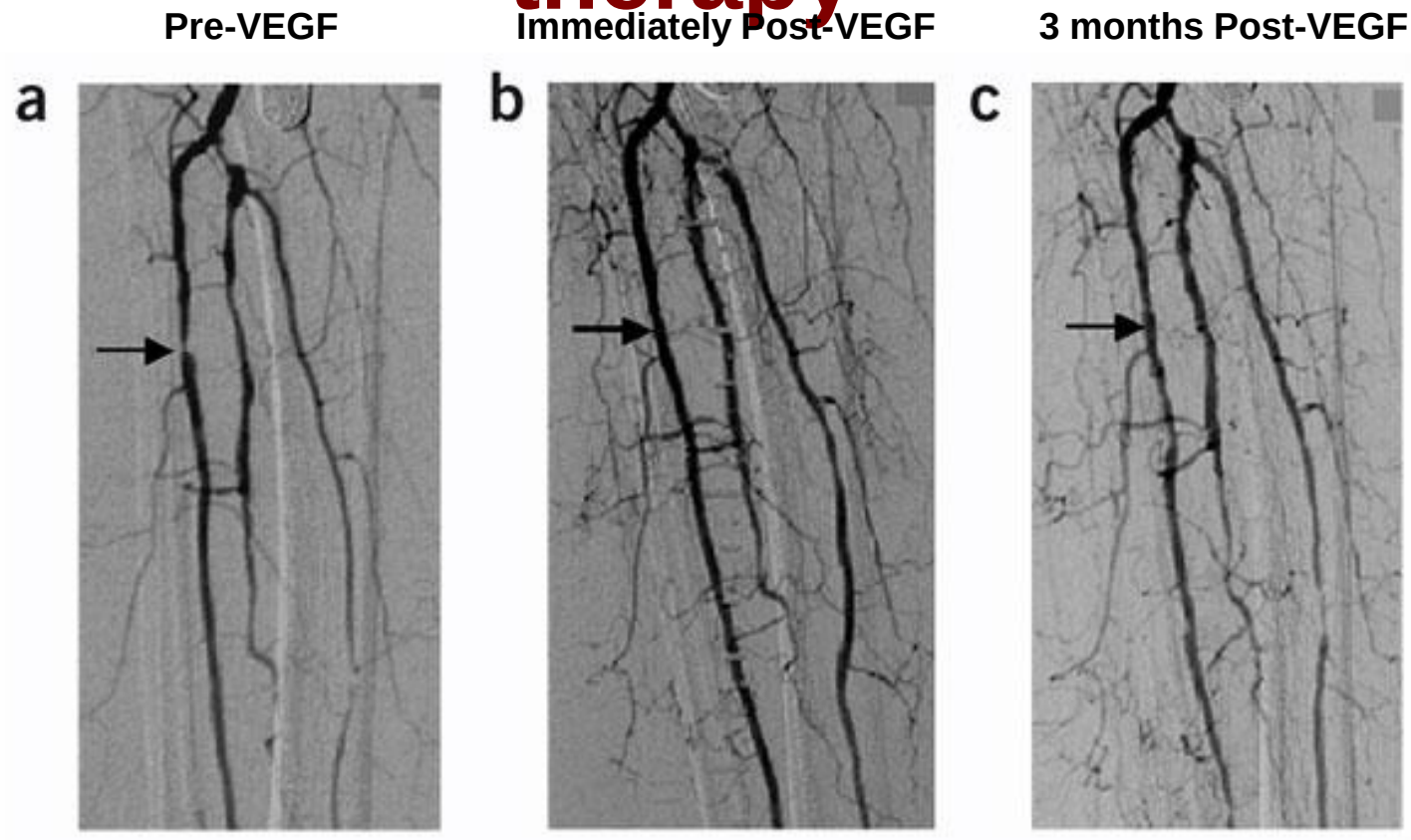
For references and more details, see Rissanen & Yla-Herttuala *Mol Ther*. 2007; 15: 1233-1247

Therapeutic angiogenesis



Courtesy of J.Lahteenvuo & Seppo Yla-Herttuala S,
University of Eastern Finland

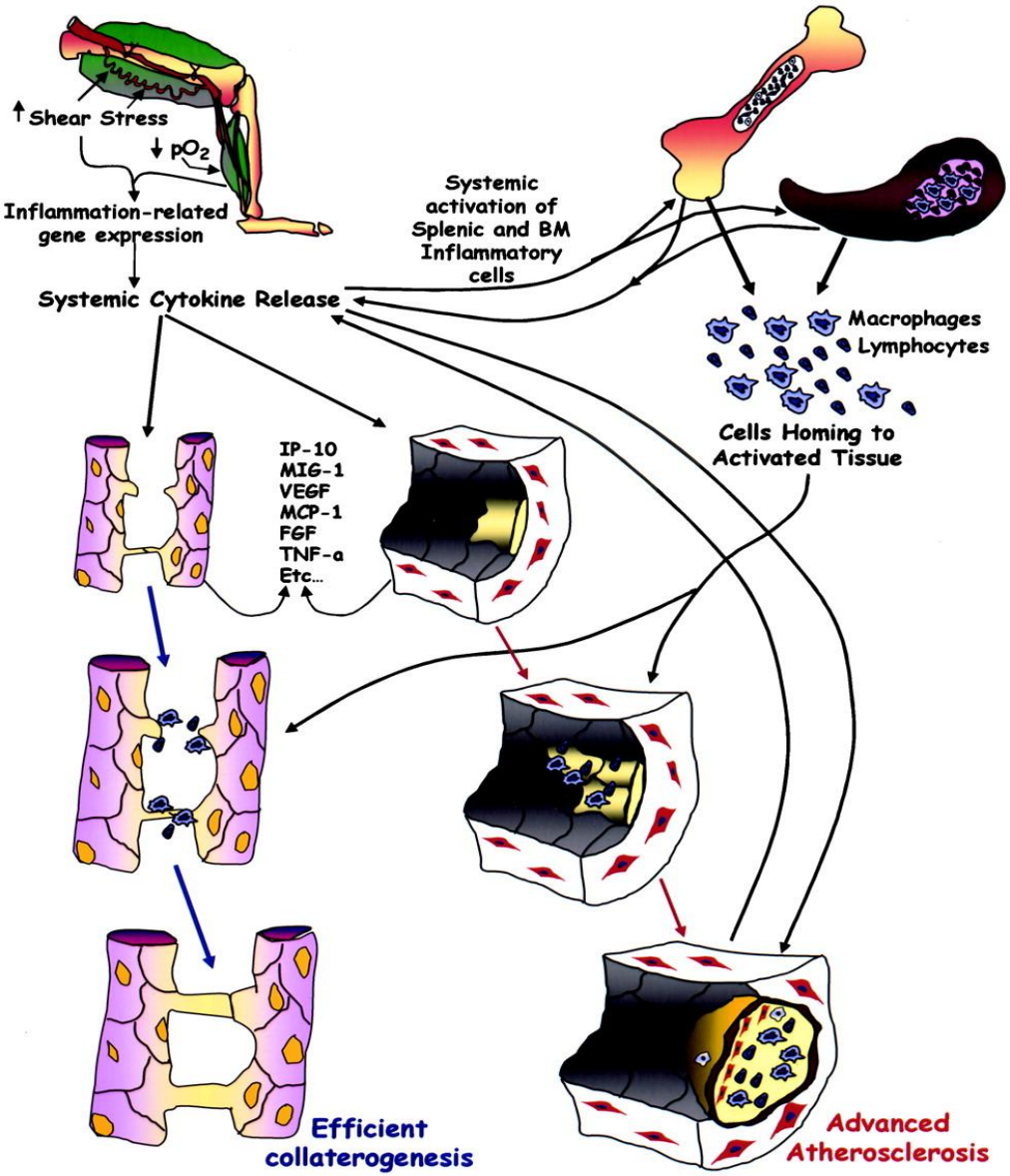
Angiography of ischaemic limb perfusion before and after VEGF gene therapy



$VEGF_{165}$ adenovirus was given by intra-arterial catheter delivery after angioplasty at the site indicated by arrows. Digital subtraction angiography was used to analyse the vasculature. (a) Before therapy. (b) Immediately after therapy. (c) Angiogram 3 months after therapy showing increased vascularisation of distal limb.

Yla-Herttuala S. & Alitalo K. *Nature Med*. 2003; 9: 694-701

Some pathways common to atherogenesis and collaterogenesis underlying the Janus phenomenon

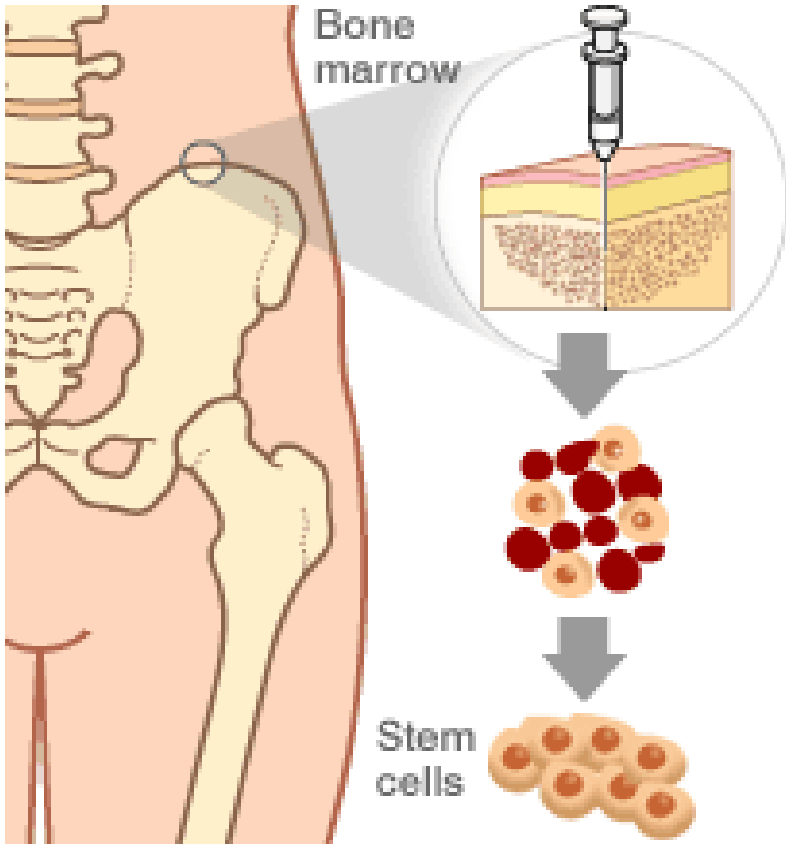


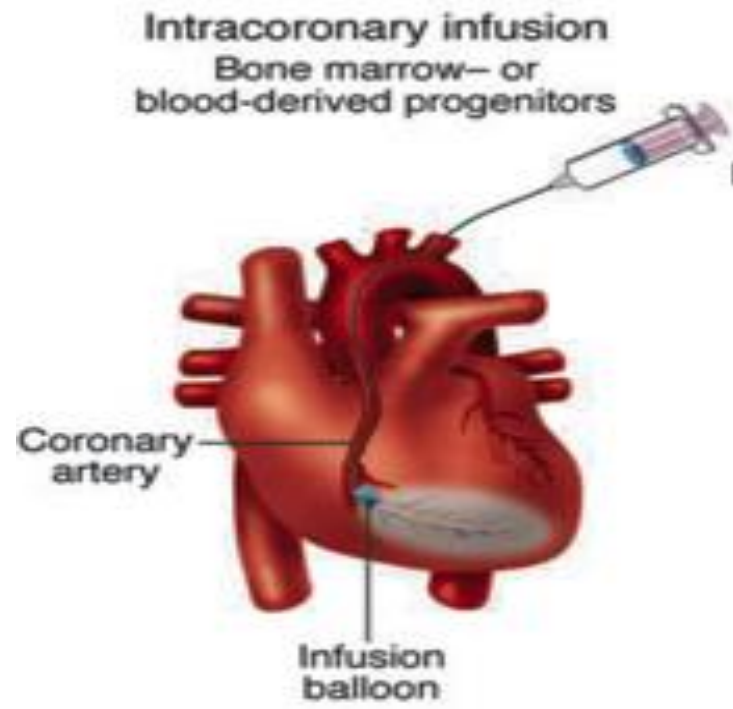
The Janus Phenomenon

Agents that enhance collateral vessel formation also increase atherogenesis and visa versa. These agents/mechanisms will activate both processes; conversely, inhibiting these mechanisms will inhibit both processes

Epstein, S. E. et al. Circulation 2004;109:2826-2831

Source and Administration of Endothelial Progenitor Cells in Acute Myocardial Infarction (AMI) Trials





Dimmeler S, Zeiher AM, Schneider MD *J Clin Invest* 2005:115;572.

Cardiovascular Side Effects of Avastin (Bevacizumab)

- **Avastin (bevacizumab; VEGF antibody) is associated with increased hypertension and thromboembolic events including myocardial infarction; haemorrhage, including gastric bleeding and nose bleeds; perforations of nose, stomach & small bowel perforation; also fatigue, high white blood cell counts**
- **Increase in strokes in wet AMD patients on high-dose Lucentis (ranibizumab) - 1.2% vs 0.3% (Hal Barron, Senior Medical Officer, Genentech 24 January 2007)**

	% patients with metastatic colorectal carcinoma	
Adverse event	**IFL; n =98**	**FL + Bevacizumab; n =109**
Proteinuria	**25.1**	**34.9**
Hypertension, all grades	**14.3**	**33.9**
Hypertension, Grade 3	**3.1**	**18.3**
Thromboembolism any	**19.4**	**13.8**
Arterial Thrombotic event	**2.0**	**4.6**
Bleeding, grade 3/4	**1.0**	**6.4**

Adapted from Hurwitz et al J Clin Oncol 2005; 23:3502-3508

Clinical phase II/III randomized controlled Therapeutic Angiogenesis Trials in Coronary and Peripheral Artery Disease: Plasmid DNA gene therapy

Trial	Disease	Therapeutic factor	Route of Administration	Control treatment	*n*	Primary endpoint	*Results	Reference
Euroinject one	CAD (CCS III–IV)	Naked $VEGF_{165}$ Plasmid	Percutaneous Intramyocardial injections	Placebo plasmid	74	Improved myocardial perfusion at 3 months	Negative	Kastrup et al JACC 2005
Genasis	CAD (CCS III–IV)	Naked VEGF-2 (VEGF-C) plasmid	Percutaneous Intramyocardial injections	Vehicle	295 (404 planned)	ETT at 3 months	Negative at interim analysis, stopped	www.medicalnewstoday.com 11 Oct 2006 (Unpublished)
Northern	CAD (CCS III–IV)	Naked $VEGF_{165}$ plasmid	Percutaneous Intramyocardial injections	Vehicle	120 (planned)	Change in myocardial perfusion in stress/rest at 12 weeks	Negative	Stewart et al Mol Ther 2009
VIF-CAD	CAD (CCS III–IV)	Naked bicistronic VEGF-A165/FGF-2 plasmid	Percutaneous Intramyocardial injections	Placebo plasmid	?	SPECT at 4 months	Ongoing	www.clinicaltrials.gov (Unpublished)
DELTA-1	PAD (claudication)	Plasmid-expressing Del-1 formulated with poloxamer 188	Intramuscular injections	Vehicle	157	PWT at 3 months	Negative	Grossman et al Am Heart J 2007
Groningen	PAD (CLI)	Naked $VEGF_{165}$ Plasmid	Intramuscular injections	Saline	54	Decrease in amputation rate	Negative (secondary endpoints positive)	Kusumanto et al Hum Gene Ther 2006
HGF-STAT	PAD (CLI)	Naked HGF plasmid	Intramuscular injections	Saline	104	Wound healing, amputation rate, rest pain, ABI	Negative	Powell et al Circulation 2008
TALISMAN 201	PAD (CLI)	Naked FGF-1 plasmid	Intramuscular injections	Vehicle	125	Ulcer healing at 6 months	Negative (secondary endpoint of reduced amputation positive)	Nikol et al Mol Ther 2008
TAMARIS	PAD (CLI)	Naked FGF-1 plasmid		Vehicle	490 (planned)	Amputation or death	Ongoing	Nikol et al Mol Ther 2008

Abbreviations: ABI, ankle brachial index; Ad, adenovirus; CLI, critical limb ischemic; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; CAD, coronary artery disease; CCS, Canadian Cardiovascular Society; PAD, peripheral arterial disease; PWT, peak walking time; SPECT, single-photon emission computed tomography; VEGF, vascular endothelial growth factor.

* Efficacy based on the defined primary or secondary endpoint.

For references and more details, see Rissanen & Yla-Herttuala *Mol Ther*. 2007; 15: 1233-1247

Clinical phase II/III randomized controlled Therapeutic Angiogenesis Trials in Coronary and Peripheral Artery Disease: Adenoviral gene therapy

Trial	Disease	Therapeutic factor	Route of Administration	Control treatment	*n*	Primary endpoint	*Results	Reference
KAT	CAD (CCS class II–III)	$AdVEGF_{165}$ or plasmid/liposome $VEGF_{165}$	Intracoronary injection at the angioplasty	Ringer's lactate	103	Improved myocardial perfusion, 6 months	Positive (AdVEGF group only)	Rajagopalan et al Circulation 2003
REVASC	CAD (CCS II–IV)	$AdVEGF_{121}$	Intramyocardial injection via mini-thoracotomy	Best medical care (no placebo)	67	Time to 1 mm ST-segment depression on ETT, 26 weeks	Positive	Stewart et al Gene Ther 2006
NOVA	CAD (CCS II–IV)	$AdVEGF_{121}$	Percutaneous Intramyocardial injections	Vehicle	129 (planned)	ETT, 26 weeks	Stopped	(Unpublished)
AGENT-2	CAD (CCS II–IV)	AdFGF-4	Intracoronary injection	Vehicle	52	SPECT, 8 weeks	Positive	Kapur & Rade Trends Cardiovasc Med 2008
AGENT-3	CAD (CCS II–IV)	AdFGF-4	Intracoronary injection	Vehicle	416	ETT, 12 weeks	Negative (subgroup of >55 yr with CCS III-IV positive)	Henry et al JACC 2007
AGENT-4	CAD (CCS II–IV)	AdFGF-4	Intracoronary injection	Vehicle	116	ETT, 12 weeks	Negative (significant beneficial effects on EET, time to angina, and CCS class in women)	Henry et al JACC 2007
AWARE	CAD (CCS III–IV)	AdFGF-4	Intracoronary injection	Vehicle	300 (women)	ETT, 6 months	Ongoing	www.clinicaltrials.gov (Unpublished)
VEGF peripheral vascular disease trial	PAD (claudication)	$AdVEGF_{165}$ or Plasmid/liposome $VEGF_{165}$	Intraarterial injection at the angioplasty	Ringer's lactate	54	Increased vascularity in angiography 3 months	Positive (Ad and plasmid groups)	Makinen et al Mol Ther 2002
RAVE trial	PAD (claudication)	$AdVEGF_{121}$	Intramuscular injections	Vehicle (no virus)	105	PWT, 12 weeks	Negative	Hedman et al Circulation 2003
WALK	PAD (claudication)	AdHIF-1/VP16	Intramuscular injections	Vehicle	300	PWT, 6 months	Ongoing	www.clinicaltrials.gov (Unpublished)

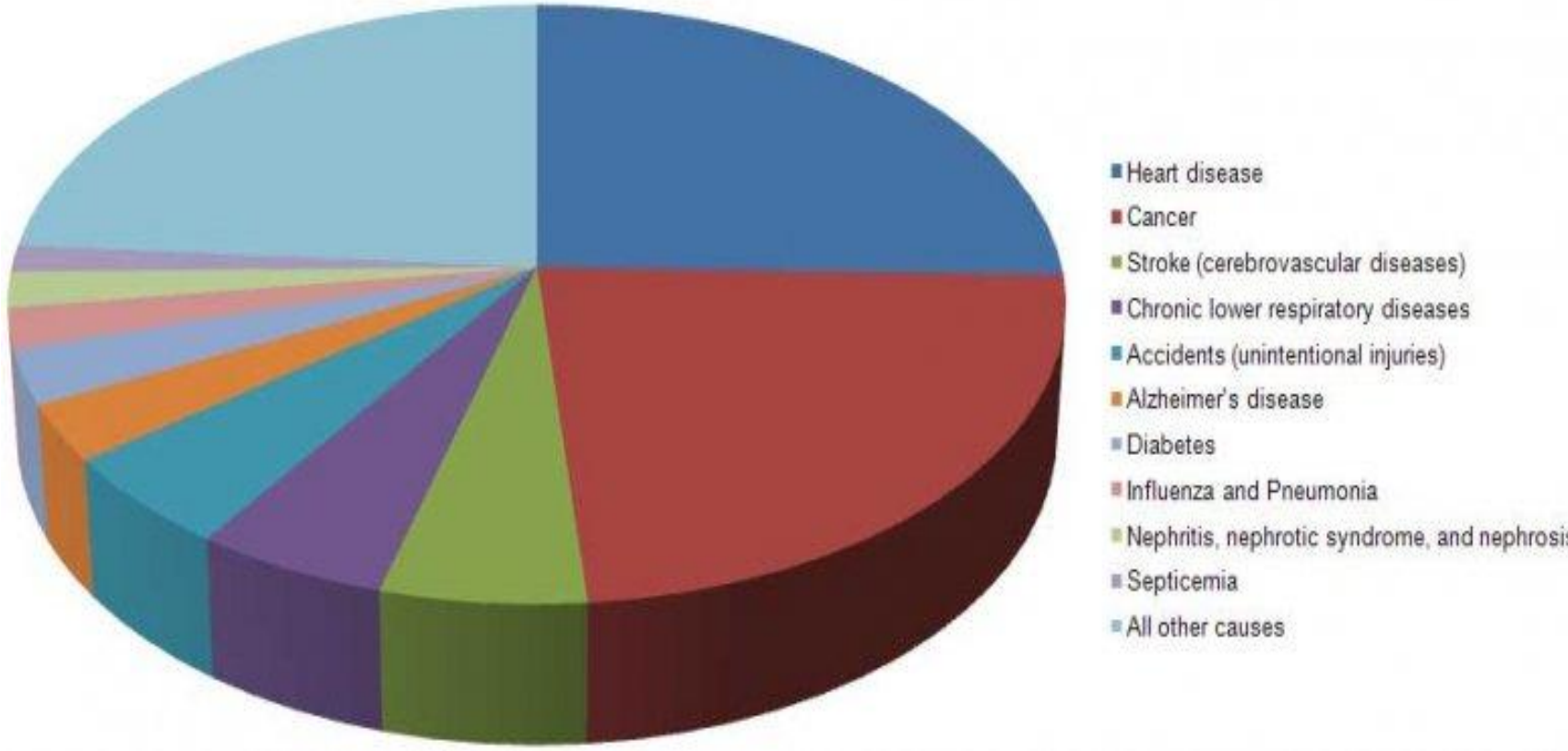
Abbreviations: Ad, adenovirus; FGF, fibroblast growth factor; HIF-1, hypoxia inducible factor-1; CAD, coronary artery disease; CCS, Canadian Cardiovascular Society; ETT, exercise tolerance test; PAD, peripheral arterial disease; PWT, peak walking time; SPECT, single-photon emission computed tomography; VEGF, vascular endothelial growth factor.

* Efficacy based on the defined primary or secondary endpoint.

For references and more details, see Rissanen & Yla-Herttuala *Mol Ther*. 2007; 15: 1233-1247

Cardiovascular disease (CVD) is the leading cause of death in western countries

USA: Cause of death in 2007



Treatment Options For Ischaemic Heart Disease

PHARMACOTHERAPY:
Combination of drugs

Antiplatelet agents

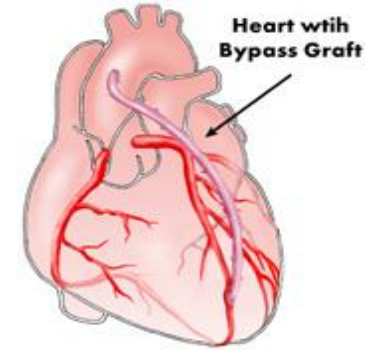
Angiotensin Converting Enzyme Inhibitors

Lipid-lowering drugs (statins)

Anti-anginal drugs (β-Blockers, Calcium antagonists)

REVASCULARISATION THERAPIES:

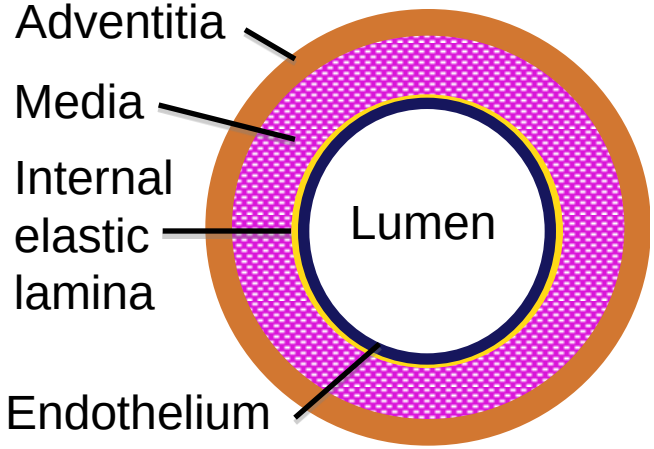
Percutaenous coronary intervention
Drug-eluting stents
Coronary artery bypass graft (CABG)



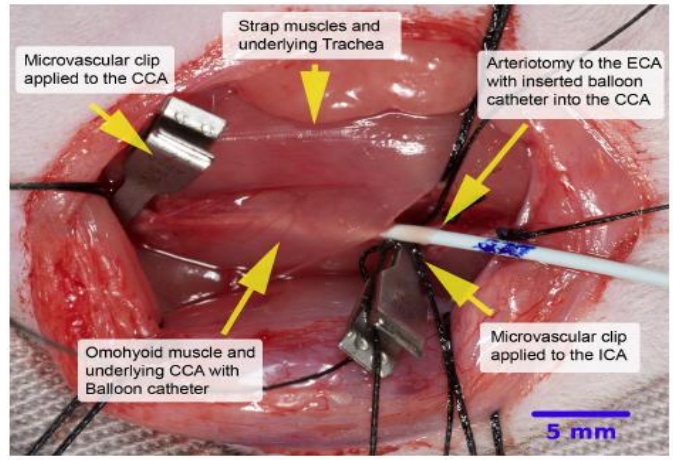
BUT

There is an increasing number of patients becoming refractory to conventional treatments!

Re-endothelialisation following balloon angioplasty

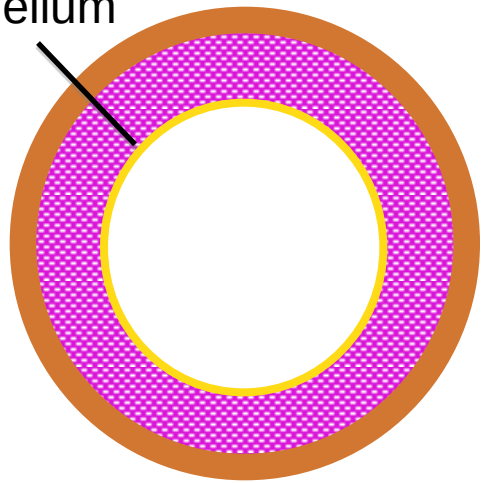


Uninjured artery



Balloon inflation to denude the endothelium

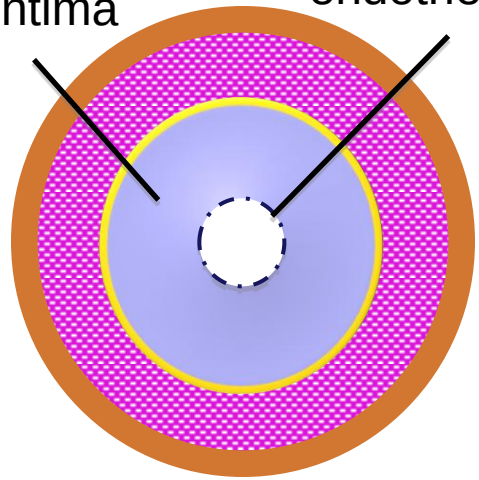
Complete loss of endothelium



1 day post injury

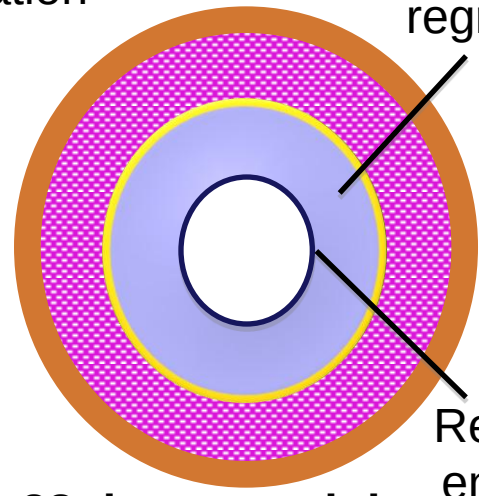
Neointima

Partial re-endothelialisation



14 days post injury

Neointimal regression



Regenerated endothelium

28 days post injury

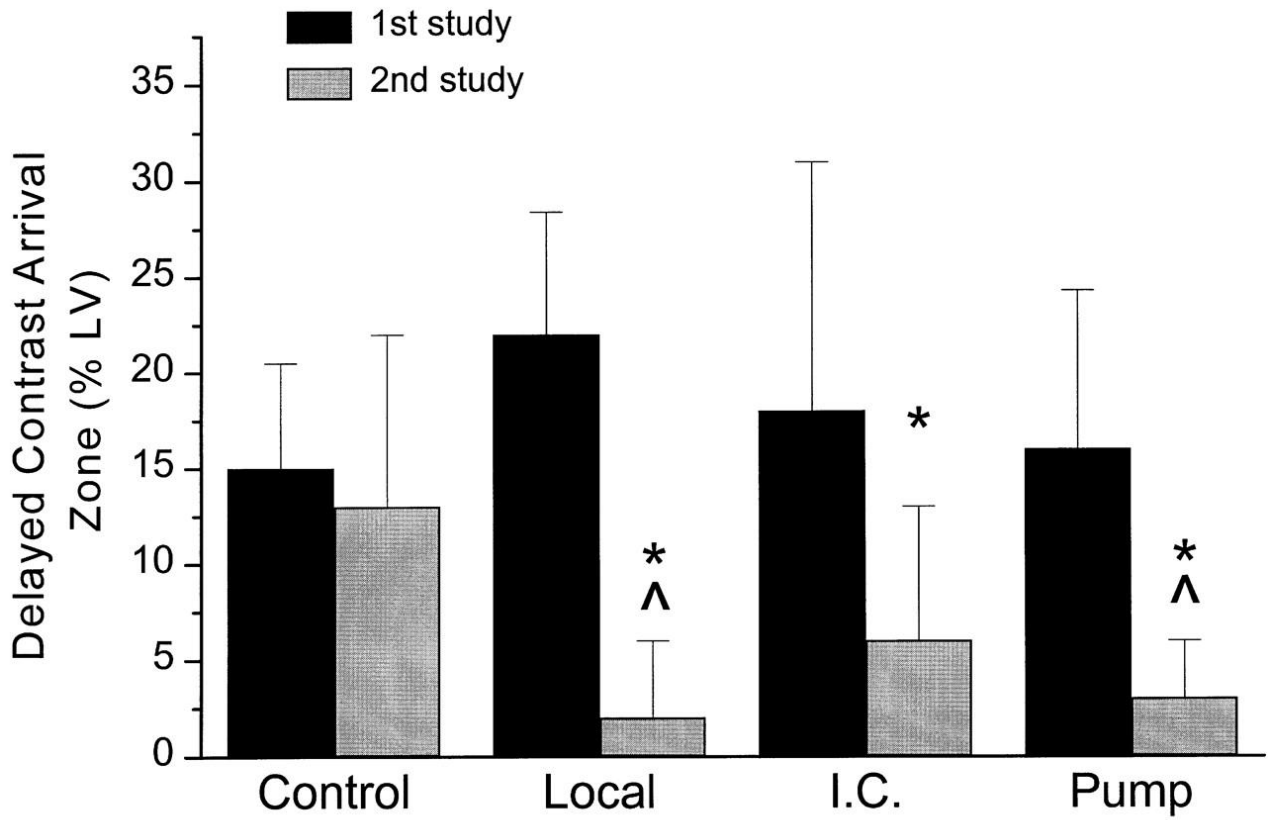
KAT301'Endocardial VEGF-D Gene Therapy for Severe Coronary Heart Disease': rationale

- Preclinical evaluation of AdVEGF-D$^{\Delta N \Delta C}$ in the pig model **of acute pig myocardial infarction** (Lähteenvuo *et al*, 2013).
- NOGA-guided **intramyocardial injection achieved efficient & localised transduction (**>50% in **1 cm^3**)
- **4-fold increase in mean capillary area up to** 21 days after gene transfer. 20% increase in ejection fraction in the AdVEGF-D$^{\Delta N \Delta C}$ treated pigs 21 days after gene transfer, as compared to the time of occlusion. Perfusion in the AdVEGF D$^{\Delta N \Delta C}$ group remained 2.8-fold higher at the infarction area 21 days after infarction.
- AdVEGF-D$^{\Delta N \Delta C}$ stimulates angiogenesis in vivo but has improved safety profile compared with AdVEGF-A: reduced effects on permeability & inflammatory cell infiltration

Therapeutic angiogenesis - practical problems

- **Many trial end points are variable, rely on subjective evaluation, are difficult to quantify and interpret, and manipulable by the placebo effect.**
- **Difficult to establish mechanism of symptomatic benefit - increased blood flow and new collateral growth - but non-invasive techniques (eg MRI, SPECT) and coronary angiography can be used.**
- **Protein versus gene therapy**
- **Delivery - site, dose, frequency, pharmacokinectics**
- **Need for larger randomized, placebo-controlled trials**

Magnetic resonance assessment of myocardial perfusion in VEGF-treated pig ameroid constrictor model.



* p <0.05 1st vs. 2nd study

^ p <0.05 vs. Control

Cardiovascular Research

John J Lopez et al. Cardiovasc Res 1998;40:272-281

Therapeutic Angiogenesis

Benefits

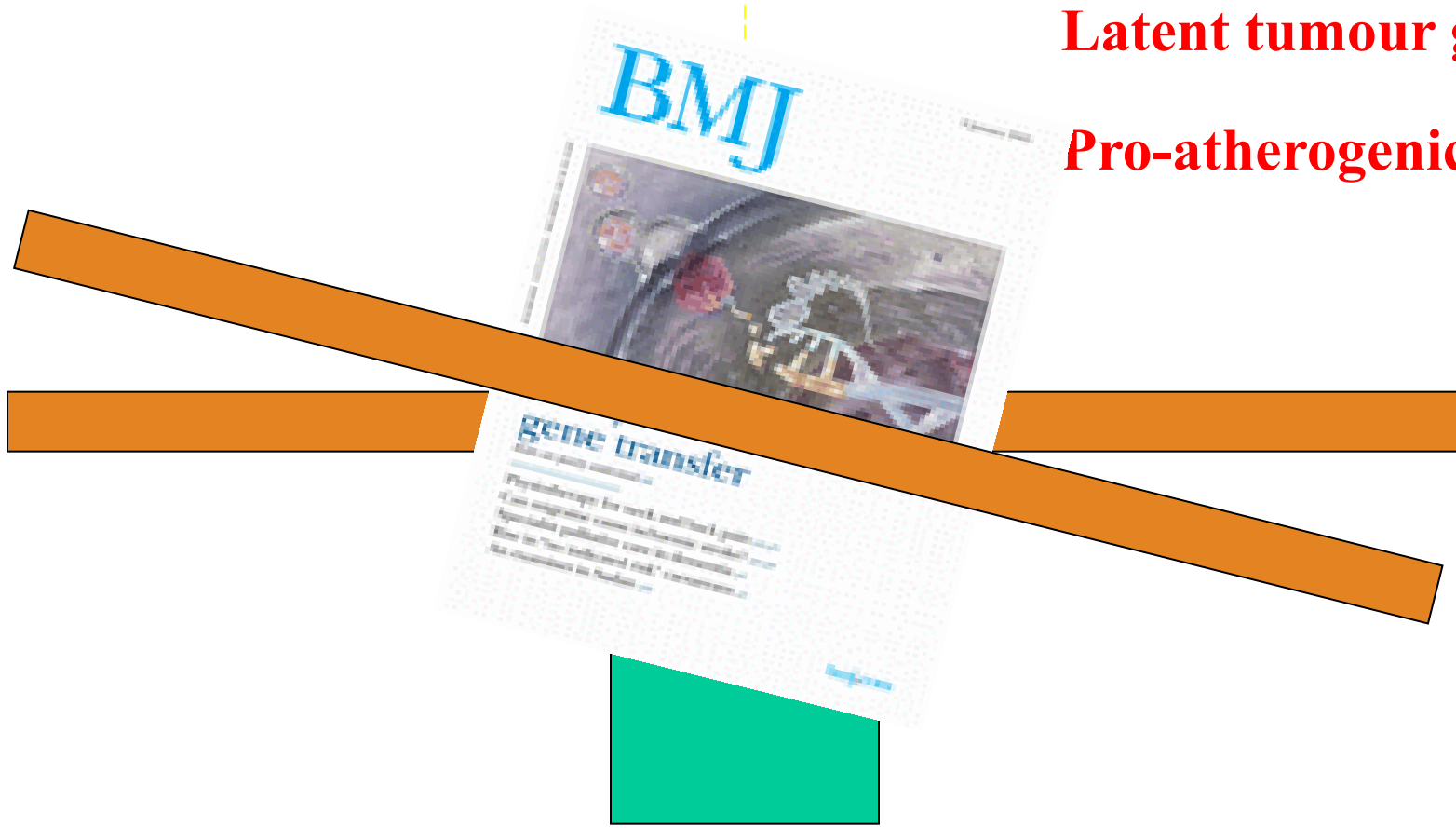
Symptomatic improvement

Concerns

Vector-associated toxicity
Abnormal vacsular growth
in non-target tissues

Latent tumour growth?

Pro-atherogenic?



Therapeutic angiogenesis – potential adverse effects

- **Increased tumour burden**
- **Local hemangioma** (Lee RJ, Springer ML, Blanco-Bose WE, Shaw R, Ursell PC, Blau HM. Circulation. 2000 Aug 22;102(8):898-901).
- **Increase in plaque rupture & arteriothromboembolic events**
- **Hypotension**

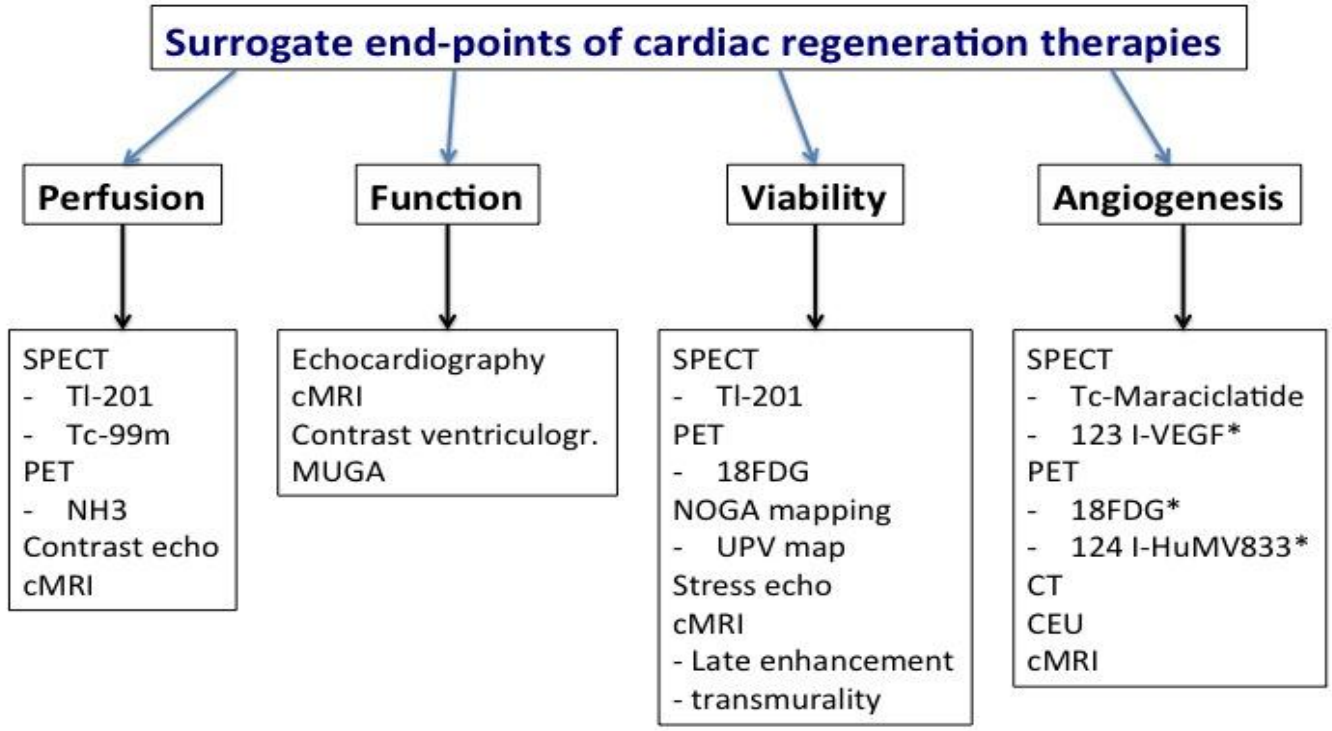
Correlation between expression of angiogenic factors in human atherosclerotic lesions and intra-plaque angiogenesis.

Growth factor / cytokine	Localization within plaque	Reference
$\alpha_v\beta_3$	Expression within macrophage foam cells	85
VEGF/VEGFR	VEGF-A, VEGF-B, VEGFR-1, and VEGFR-2 staining evident within plaque SMCs	37
FGF2	Secreted by intraplaque mast cells	86
PD-ECGF	Expression within plaque macrophages and ECs of plaque neovessels, from coronary atherectomy specimens.	87
PAF	Expression correlated with CD68+ve monocytes	88
PDGF-A and-B	Expression correlated with SMCs and macrophages	89
HGF	Expression correlated with carotid atherosclerotic plaques, but not normal arteries	90
TGFβ1	Expression with activated macrophages, T lymphocytes and SMCs	91
HB-EGF	Macrophages and SMCs	92
IL-8	Protein and mRNA present within direct coronary atherectomy homogenates	49
tPA, uPA	Intimal SMCs, macrophage-derived foam cells and plaque neo-vessels	93

PD-ECGF, Platelet-derived endothelial cell growth factor; PAF, Platelet activating factor; PDGF, Platelet-derived growth factor; HGF, Hepatocyte growth factor; TGF, Transforming growth factor; HB-EGF, Heparin-binding Epidermal growth factor-like growth factor; IL-8, Interleukin-8; t/u PA, tissue/urokinase-type plasminogen activator.

Khurana et al Circulation 2005; 112: 1813-1824

KAT301'Endocardial VEGF-D Gene Therapy for Severe Coronary Heart Disease': Secondary end-points



Adapted from Fuster et al. Nat Clin Pract Cardiovasc Med 2007

Comparison of different types of neovascularization

	Vasculogenesis	Angiogenesis	Arteriogenesis
Cell types	Endothelial stem cells	Endothelial cells	Endothelial cells, SMC, Pericytes, monocytes, other?
Stimulus	Development,	Ischaemia, inflammation, development, disease	Development; stimulus for collateralization in adult heart unclear
Result	Differentiated Endothelial cells, primitive Vascular system	Capillaries	Arterioles, arteries
Occurrence in adult	Unclear	Yes	Yes
Contribution to effective perfusion	Unclear	Minor	Major
Growth factors	VEGFA and C, FGF-1, FGF-2, FGF-4, FGF-5	VEGFA and C, FGF-1, FGF-2, FGF-4, FGF-5	PDGF, Ang-1, Ang-2, FGFs, MCP-1, Ephrins

Stem and Progenitor Cell Therapy: Unresolved Issues

- **Stem cells/EPCs are not fully defined**
- **Mechanisms unclear: eg transdifferentiation of bone marrow-derived progenitors in ischaemic heart versus secretion of soluble factors; collateral formation versus prevention of cardiomyocyte apoptosis**
- **Do cardiovascular risk factors and disease impair efficacy of EPCs?**
- **Some trials negative; evidence from large RCTs lacking**

VEGF inhibitor: Bevacizumab (Avastin)

- **Humanised monoclonal anti-VEGF antibody developed by Genentech Inc**
- **Inhibits VEGF-induced angiogenesis and tumour growth in vivo (PoC)**
- **Effective in combination with first-line cytotoxic drug treatment (chemotherapy; eg 5-fluorouracil)**
- **In Phase III trials for metastatic colon cancer, bevacizumab increased time to disease progression when administered in combination with conventional chemotherapy, compared to chemotherapy alone**
- **February 2004: Approved by US Food and Drug Administration (FDA) as adjunct to first-line cytotoxic treatment of metastatic colorectal cancer (in combination with 5-fluorouracil)**
- **Now also approved for non-small cell lung carcinoma, glioblastoma and renal cancer (also in combination with chemotherapy). Trials underway in other cancers. Approval of Avastin for breast carcinoma withdrawn by FDA late 2011.**

Recommended reading

Therapeutic Angiogenesis for Cardiovascular Disease

Henry et al. The VIVA Trial. Vascular endothelial growth factor in ischemia for vascular angiogenesis. *Circulation* 2003;107:1359-1365

Rissanen TT, Ylä-Herttuala S. Current status of cardiovascular gene therapy. *Mol Ther.* 2007;15:1233-47.

Khurana et al The Role of Angiogenesis in Cardiovascular Disease: A Critical Appraisal. *Circulation* 2005; 112: 1813-24.

Zachary IC and Morgan R. Therapeutic angiogenesis for cardiovascular disease: biological context, challenges, prospects. *Heart* 2011; 97:181-9.

Zachary et al. Vascular Protection: A Novel Nonangiogenic Cardiovascular Role for VEGF. *Arterioscler Thromb Vasc Biol* 2000; 20: 1512-1520.

Barger et al. Hypothesis - Vasa Vasorum and Neovascularization of Human Coronary-Arteries - A Possible Role in the Patho-Physiology of Atherosclerosis. *New England Journal of Medicine* 1984;310:175-177

Epstein et al. Janus Phenomenon: the interrelated tradeoffs inherent in therapies designed to enhance collateral formation and those designed to inhibit atherogenesis. *Circulation*. 2004;109:2826-31.

Stem cell therapy for Cardiovascular Disease

Mollmann et al. Stem cells in myocardial infarction: from bench to bedside. *Heart*, 2009; 95: 508 - 514.

Martin-Rendon et al. Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. *Eur Heart J*. 2008;29:1807-18.

Nowbar, A. N. *et al*. Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. Br. Med. J. 348, g2688 (2014).

Laflamme, M. A. & Murry, C. E. Heart regeneration. Nature 473, 426–335 (2011).

Bolli, R. *et al*. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 378, 1847–1857 (2011).

Bartunek, J. *et al*. Cardiopoietic Stem Cell Therapy in Heart Failure : The C-CURE (Cardiopoietic stem Cell therapy in heart failURE) Multicenter Randomized Trial With Lineage-Specified Biologics. J. Am. Coll. Cardiol. 61, 2329–2338 (2013)

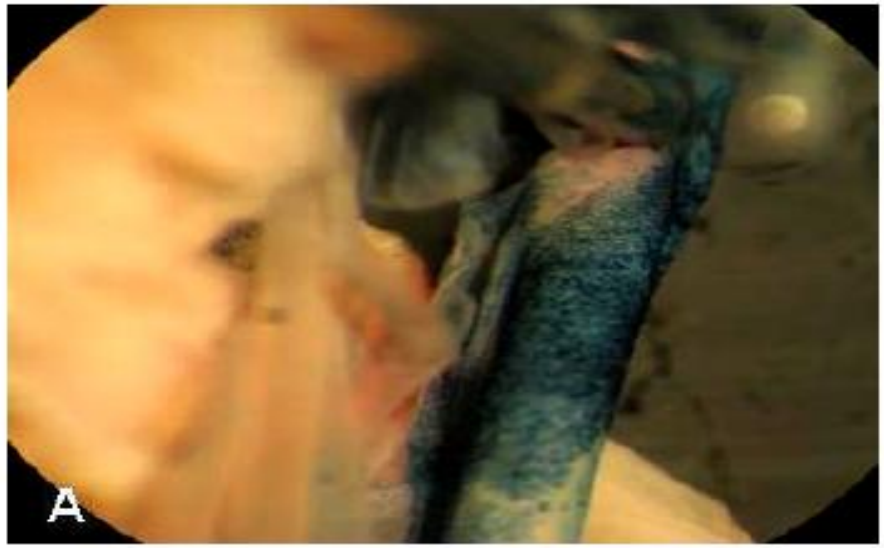
Apparent Janus-Like Effects of Cells on Atherogenesis and Collaterogenesis

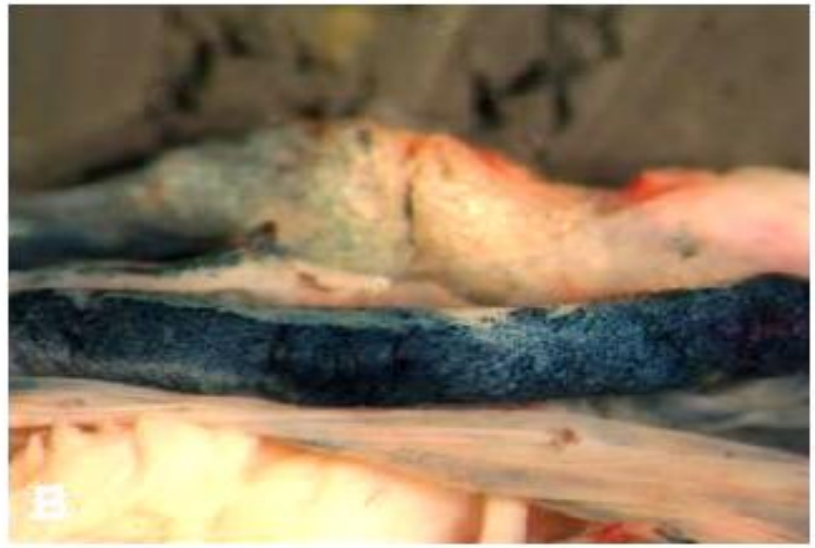
	Atherogenesis	Collaterogenesis
BM-derived MNC	Increases,[70] inhibits[71]	Increases[65–69]
Monocyte/macrophage	Increases[10,23,53]	Increases[10]
T cells	Increases[23,53,72,73]	Increases[28]
Mast cells	Increases[53,74]	Increases[75]

BM-derived MNC indicates bone marrow-derived mononuclear cells.

Epstein et al Circulation. 2004;109:2826-31

Administration of Ad.LacZ ($1x10^{10}$ vp) to the utero-placental vessels:

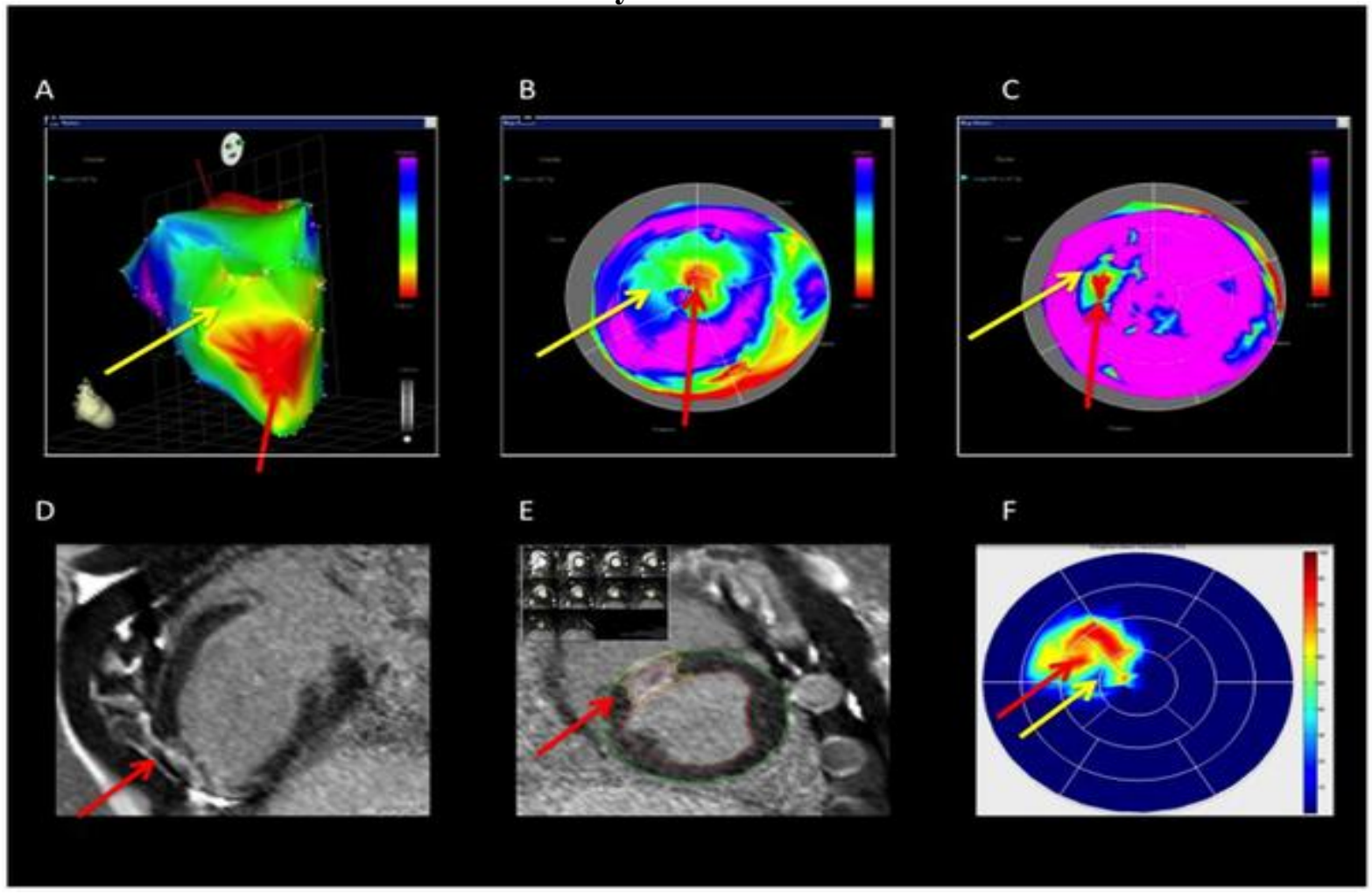




Statistical Analysis:

Generalized Mixed Linear Model, accounting for Fetal Sex, Uterine Position, Litter Number and Treatment

Figure 1. NOGA endocardial mapping and cardiac magnetic resonance imaging (cMRI) of a pig with chronic myocardial ischemia.



Pavo N, Jakab A, Emmert MY, Strebinger G, Wolint P, et al. (2014) Comparison of NOGA Endocardial Mapping and Cardiac Magnetic Resonance Imaging for Determining Infarct Size and Infarct Transmurality for Intramyocardial Injection Therapy Using Experimental Data. PLoS ONE 9(11): e113245. doi:10.1371/journal.pone.0113245
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0113245

KAT301‘Endocardial VEGF-D Gene Therapy Trial: NOGA catheter-based targeted delivery to the ischemic but viable myodardium

- Cardiac magnetic resonance imaging (cMRI) with late gadolinium enhancement (LE) is the gold standard for assessing myocardial infarct size, infarct transmurality, and LV function and for assessing the efficacy of cardiac therapies but is an off-line imaging modality, and there is a delay between diagnostic imaging and application of the therapy when the patient is in the cath lab.

- The NOGA (Hebrew for sparkle or brightness, and the planet Venus) system allows real-time 3D nonfluoroscopic electromechanical mapping of the myocardium and is the only technique that enables nonfluoroscopic mapping of the heart and 3D navigation during percutaneous intramyocardial delivery of regenerative therapies.

- The border zone of myocardial infarction (MI) represents myocardial areas with decreased viability and reduced wall motion capacity (hibernating, myocardium). NOGA recognizes preserved electrical activity indicating hibernating myocardium. Because they are viable, regenerative therapies delivered to these areas may better restore cardiac function.

- Catheter-based direct intramyocardial injection may have several advantages over other delivery routes: 1) reduced likelihood of systemic toxicity and unwarranted hemodynamic effects of the injected substance, 2) minimal wash-out, resulting in limited exposure of non-target organs, 3) high degree of intramyocardial accumulation of the delivered vectors, allowing a reduced applied dose, 4) precise localization to ischemic and peri-ischemic myocardial regions, 5) treatment of myocardial areas with completely occluded epicardial vascular beds, and 6) avoiding ischemia induced by coronary intervention (M. Gyöngyösi et al. Nature Rev Cardiol 2011).

- Diagnostic (identification of stunned and hibernating myocardium, distinguishing viable from non-viable cardiac tissue) and prognostic (determination of infarct transmurality) value of the diagnostic NOGA mapping

Diseases characterized or caused by insufficient angiogenesis or lymphangiogenesis

Organ	Disease
Blood and lymph vessels	Atherosclerosis, restenosis, diabetes, hypertension
Heart	Ischaemic heart disease, cardiac failure
Periphery (lower leg)	Ischaemic disease
Nervous system	Stroke; Alzheimer's disease; Diabetic neuropathy; Amyotrophic lateral sclerosis (ALS)
Gastrointestinal tract	Gastric or oral ulcerations; Crohn's disease
Skin	Hair loss; Skin purpura, telangiectasia, and venous lake formation; Systemic sclerosis, Lupus
Bone, joints	Osteoporosis, impaired bone fracture healing
Lung	Neonatal respiratory distress syndrome (RDS); Pulmonary fibrosis, emphysema
Kidney	Nephropathy (ageing; metabolic syndrome); glomerulosclerosis; tubulointerstial fibrosis
Reproductive system	Preeclampsia; Intrauterine growth retardation; Menorrhagia (uterine bleeding)

Therapeutic angiogenesis: Is there still unmet clinical need?

- **United Kingdom: in 2000 634,000 angina sufferers consulted GPs 2.35 million times costing £60.5m. They required 16 million prescriptions (£80.7m) and 254,000 hospital outpatient referrals (£30.4m). There were 149,000 hospital admissions, 117,000 coronary angiograms, 21,400 coronary artery bypass operations, 17,700 percutaneous coronary interventions, and 516,000 outpatient visits, costing £208.4m, £69.9m, £106.2m, £60.7m, and £52.2m, respectively. The direct cost of angina was therefore £669m (€815m) or 1.3% of total NHS expenditure. (S. Stewart *et al;* Heart: 2003;89:848–853).**
- **2.5-7.5% of patients with CHD remain refractory to the standard therapy. 5-10% of patients undergoing cardiac catheterization have refractory angina, >1.8 million Americans and 500000 Canadians have refractory angina (Henry T et al, Nat Rev Cardiol 2014: 11:78-95), and ~100,000 new refractory angina sufferers identified in Europe each year (S. Eldabe, Trials 2013;14:57).**

Clinical trials of Therapeutic Angiogenesis in Coronary Artery Disease: Protein Therapy

Therapeutic factor	Trial type	n	Route of administration	Results/Effects	Reference
FGF1	Phase I,	20	IM injection	Safe; Capillary blush at injection site	41
FGF2	Phase I/II DBR	24	Heparin–alginate	Reduced ischemic zone size; Effect sustained at 3 years	42,43
FGF2	Phase I,	52	IC infusion	Improved symptoms; Reduced SPECT defect size; hypotension at high dosages	44
FGF2	Phase I,	30	IC infusion	Hypotension at high dosages; dilatation of epicardial coronaries	45,46
FGF2	Phase II, DBR	337	IC infusion	Safe; No effect on ETT or SPECT; Short-term improvement in symptoms compared to placebo	47
VEGF-A_{165}	Phase I,	15	IC infusion	Reduced SPECT defect size; Hypotension at low dosages	48
VEGF-A_{165}	Phase I,	14	IV infusion	Safe; No clear effects	49
VEGF-A_{165}	Phase II, DBR	165	IC+IV infusion	No improvement in ETT, symptoms, or SPECT compared to controls	50
GM-CSF	Phase I/II DBR	21	IC+IV 2 wk infusion	Improved collateral flow index in the GM-CSF group	51

Abbreviations: DBR, double-blind, randomised; ETT, exercise tolerance test; FGF, fibroblast growth factor; GM-CSF, Granulocyte/Monocyte Colony Stimulating factor; IC, intracoronary; IV, intravenous; IM, intramyocardial; SPECT, single-photon emission computed tomography; VEGF, vascular endothelial growth factor.

* Efficacy based on the defined primary or secondary endpoint.

For references and more details, see Annex &
Simons *Cardiovasc Res*. 2005; 65: 649-655

VEGF (VEGF-A) is essential for developmental and disease-linked angiogenesis

- **1983 - Tumours secrete a Vascular permeability Factor**
- **1989 - Cloning of VEGF-A**
- **Hypoxia-inducible secreted polypeptide – Vascular Endothelial Growth Factor (VEGF-A); also called Vascular Permeability Factor (VPF)**
- **Specific mitogen, chemoattractant and survival factor for endothelial cells. Permeability increasing factor, increases vasodilatation and hypotension in vivo**
- **1990s: Experimental evidence that VEGF-A is essential for tumour growth. VEGF is upregulated and regulates angiogenesis in many tumours and other neovascularizing diseases. Inhibition of VEGF-A and of VEGFR2 (Flk-1) inhibits tumour growth in vivo (Kim et al, *Nature* 1993; Millauer et al Nature 1994)**
- **Loss of one copy of the VEGF-A gene causes aberrant blood vessel formation and death in embryogenesis (Ferrara et al *Nature* 1996; Carmeliet et al *Nature* 1996). Disruption of genes for VEGF-R2 (KDR/Flk-1) (Shalaby et al *Nature* 1995) or VEGF-R1 (Flt-1) (Fong et al *Nature* 1995) is embryonic lethal due to aberrant blood vessel formation**
- **1990s – Experimental evidence that VEGF-A is essential for tumour growth: Many human tumours express VEGF-A. Animal models show the importance of VEGF-A for developmental and disease-related angiogenesis (Ferrara & co-workers, Genentech).**
- **February 2004 – Translation to the clinic: FDA approves anti-VEGF antibody, bevacizumab (Avastin) for metastatic colorectal cancer (Genentech)**