Therapeutic Angiogenesis: The Complexities of Therapeutic Translation

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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

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

1983: Vascular permeability factor discovered

1989-1991: VEGF-A cloned & protein purified

1990s: VEGF upregulated by hypoxia & in many neoplasms

1993: anti-VEGF inhibits tumours in mice (Kim et al Nature)

1993-6: VEGFR and VEGF-A KOs inhibit embryonic vascularisation

1983: Results of 1st RCT for VEGF in Ischaemic heart disease

2004: US FDA approves Avastin for metastatic colorectal cancer

2003: Results of 1st RCT for VEGF in Ischaemic heart disease

2006 FDA approves anti-VEGF (Lucentis) for wet AMD

2006: FDA approves sVEGF-Fc (Eylea) for wet AMD

2011: FDA cancels approval for Avastin in breast cancer
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\textsubscript{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-endothelialisation

Cardiac & peripheral ischaemic disease:
Therapeutic angiogenesis

Angioplasty, stenting or CABG: Vascular protection
<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>VEGF</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limb ischemia</td>
<td>Rabbit hindlimb</td>
<td>Protein intravenous</td>
<td>Increased revascularization(^{13})</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Recovery of collateral endothelium-dependent flow(^{16})</td>
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<td></td>
<td></td>
<td>Protein intravenous</td>
<td>Evidence of enhanced collateral formation(^{114})</td>
</tr>
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<td></td>
<td>Increased cell proliferation and collateral formation(^{115})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cDNA intramuscular</td>
<td>Improved muscular blood supply(^{18})</td>
</tr>
<tr>
<td>Rat hindlimb</td>
<td></td>
<td></td>
<td>Increased collateral supply(^{117})</td>
</tr>
<tr>
<td>Canine hindlimb</td>
<td></td>
<td>Protein intravenous</td>
<td>Evidence of enhanced collateral formation(^{116})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Improved muscular blood supply and increased capillary density(^{118})</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Porcine</td>
<td>Protein intravenous</td>
<td>Increased collateral-dependent flow and hypotension(^{20})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased coronary flow(^{19})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protein intracoronary</td>
<td>Increased collateral blood supply shown by MRI(^{15})</td>
</tr>
<tr>
<td>Canine</td>
<td></td>
<td>Protein intravenous</td>
<td>Improved vasomotor responses(^{119})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cDNA (VEGF(_{121}))</td>
<td>Increased angiogenesis(^{21})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Collateral formation and increased perfusion(^{22})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased angiogenesis and collateral flow(^{14})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protein intravenous</td>
<td>No increase in collateral formation and increased neointimal hyperplasia(^{37})</td>
</tr>
<tr>
<td>Balloon injury</td>
<td>Rat carotid</td>
<td>Protein intravenous</td>
<td>Accelerated reendothelialization(^{9})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cDNA intravenous</td>
<td>Accelerated reendothelialization(^{10})</td>
</tr>
<tr>
<td>Stent implantation</td>
<td>Rabbit iliac</td>
<td>Protein intravenous</td>
<td>Accelerated reendothelialization, decreased neointima formation and reduced mural thrombosis(^{11})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cDNA intravenous</td>
<td>Accelerated reendothelialization, decreased neointima formation and reduced mural thrombosis(^{12})</td>
</tr>
<tr>
<td>Vein graft</td>
<td>Rabbit</td>
<td>Protein topical</td>
<td>Decreased neointima formation(^{24})</td>
</tr>
<tr>
<td>Extravascular</td>
<td>Rabbit carotid</td>
<td>cDNA local extravascular</td>
<td>Reduced neointima formation in presence of intact endothelium and absence of angiogenesis(^{55})</td>
</tr>
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<td>silastic collar</td>
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Gene Delivery to the Cardiovascular System

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

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 rhVEGF.

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

Placebo n=63
17 ng/kg/min n=56  rhVEGF$_{165}$: IC + IV x 3
50 ng/kg/min n=59

Efficacy endpoints (60 and 120 days)

Treadmill time
Clinical
SPECT - myocardial perfusion
Angiography

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

Vascular protection - Essential for vascular maintenance

Physiological (Therapeutic) Angiogenesis

Pathophysiological Angiogenesis - chaotic, leaky vasculature

Dose matters: Concentration-dependent

Effect of VEGF in the Cardiovascular System

NO, PGI₂
Increased blood flow, Cell survival

Cell migration, proliferation, morphogenesis

Increased permeability

VEGF concentration

Biological effect

ENDOTHELIAL CELL

TISSUE
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.

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

EC assembly

Stabilisation

Maturation

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

- Increased vascular permeability
- Tumour angiogenesis
- Neovascular eye disease
- Hypotension
- Increase in plaque rupture & heart attack
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.


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

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Alex Fantin, Christiana Ruhrberg

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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$^{ΔNΔC}$ in the pig model of acute pig myocardial infarction (Lähteenvuori et al, 2013).
- AdVEGF-D$^{ΔNΔC}$ 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$^{ΔNΔC}$) 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
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.
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

<table>
<thead>
<tr>
<th>Therapeutic application</th>
<th>Desired biological effect</th>
<th>Therapeutic factor</th>
<th>Target for Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promotion of growth of blood vessels and lymphatic vessels</td>
<td>Stimulation of capillary growth (angiogenesis), collateral artery growth (arteriogenesis) and lymphatic vessel growth (lymphangiogenesis)</td>
<td>VEGF (VEGF-A), PiGF, 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</td>
<td>Ezrin</td>
</tr>
<tr>
<td>Vascular protection, and prevention of restenosis, in-stent restenosis, and graft failure</td>
<td>Enhanced endothelial function (vasodilatation and anti-thrombosis), endothelial regeneration/repair, reduced SMC proliferation and migration, anti-inflammation, inhibition of excess matrix production, induction of apoptosis</td>
<td>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</td>
<td>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</td>
</tr>
</tbody>
</table>

**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; PiGF, 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.
Therapeutic angiogenesis
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.

Some pathways common to atherogenesis and collateralogenesis 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

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)

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>IFL; n =98</th>
<th>FL + Bevacizumab; n =109</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria</td>
<td>25.1</td>
<td>34.9</td>
</tr>
<tr>
<td>Hypertension, all grades</td>
<td>14.3</td>
<td>33.9</td>
</tr>
<tr>
<td>Hypertension, Grade 3</td>
<td>3.1</td>
<td>18.3</td>
</tr>
<tr>
<td>Thromboembolism any</td>
<td>19.4</td>
<td>13.8</td>
</tr>
<tr>
<td>Arterial Thrombotic event</td>
<td>2.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Bleeding, grade 3/4</td>
<td>1.0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

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

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

*Abbreviations: ABI, ankle brachial index; Ad, adenovirus; CLI, critical limb ischemic; FGF, fibroblast 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.
Clinical phase II/III randomized controlled Therapeutic Angiogenesis Trials in Coronary and Peripheral Artery Disease: Adenoviral gene therapy

<table>
<thead>
<tr>
<th>Trial</th>
<th>Disease</th>
<th>Therapeutic factor</th>
<th>Route of Administration</th>
<th>Control treatment</th>
<th>n</th>
<th>Primary endpoint</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAT</td>
<td>CAD (CCS class II-III)</td>
<td>AdVEGF&lt;sub&gt;145&lt;/sub&gt; or plasmid/liposome VEGF&lt;sub&gt;160&lt;/sub&gt;</td>
<td>Intracoronary injection at the angioplasty</td>
<td>Ringer's lactate</td>
<td>103</td>
<td>Improved myocardial perfusion, 6 months</td>
<td>Positive (AdVEGF group only)</td>
<td>Rajagopalan et al. Circulation 2003</td>
</tr>
<tr>
<td>REVASC</td>
<td>CAD (CCS II-IV)</td>
<td>AdVEGF&lt;sub&gt;121&lt;/sub&gt;</td>
<td>Intramyocardial injection via mini-thoracotomy</td>
<td>Best medical care (no placebo)</td>
<td>67</td>
<td>Time to 1 mm ST-segment depression on ETT, 26 weeks</td>
<td>Positive</td>
<td>Stewart et al. Gene Ther 2006</td>
</tr>
<tr>
<td>NOVA</td>
<td>CAD (CCS II-IV)</td>
<td>AdVEGF&lt;sub&gt;121&lt;/sub&gt;</td>
<td>Percutaneous Intramyocardial injections</td>
<td>Vehicle injections</td>
<td>129 (planned)</td>
<td>ETT, 26 weeks</td>
<td>Stopped</td>
<td>(Unpublished)</td>
</tr>
<tr>
<td>AGENT-3</td>
<td>CAD (CCS II-IV)</td>
<td>AdFGF-4</td>
<td>Intracoronary injection</td>
<td>Vehicle</td>
<td>416</td>
<td>ETT, 12 weeks</td>
<td>Negative (subgroup of &gt;55 yr with CCS III-IV positive)</td>
<td>Henry et al. JACC 2007</td>
</tr>
<tr>
<td>AGENT-4</td>
<td>CAD (CCS II-IV)</td>
<td>AdFGF-4</td>
<td>Intracoronary injection</td>
<td>Vehicle</td>
<td>116</td>
<td>ETT, 12 weeks</td>
<td>Negative (significant beneficial effects on ETT, time to angina, and CCS class in women)</td>
<td>Henry et al. JACC 2007</td>
</tr>
<tr>
<td>AWARE</td>
<td>CAD (CCS III-IV)</td>
<td>AdFGF-4</td>
<td>Intracoronary injection</td>
<td>Vehicle</td>
<td>300 (women)</td>
<td>ETT, 6 months</td>
<td>Ongoing</td>
<td><a href="http://www.clinicaltrials.gov">www.clinicaltrials.gov</a> (Unpublished)</td>
</tr>
<tr>
<td>VEGF peripheral vascular disease trial</td>
<td>PAD (claudication)</td>
<td>AdVEGF&lt;sub&gt;144&lt;/sub&gt; or Plasmid/liposome VEGF&lt;sub&gt;160&lt;/sub&gt;</td>
<td>Intrarterial injection at the angioplasty</td>
<td>Ringer's lactate</td>
<td>54</td>
<td>Increased vascularity in angiography 3 months</td>
<td>Positive (Ad and plasmid groups)</td>
<td>Mäkinen et al. Mol Ther 2002</td>
</tr>
<tr>
<td>RAVE trial</td>
<td>PAD (claudication)</td>
<td>AdVEGF&lt;sub&gt;121&lt;/sub&gt;</td>
<td>Intramuscular injections</td>
<td>Vehicle (no virus)</td>
<td>105</td>
<td>PWT, 12 weeks</td>
<td>Negative</td>
<td>Hedman et al. Circulation 2003</td>
</tr>
<tr>
<td>WALK</td>
<td>PAD (claudication)</td>
<td>AdHIF-1b/VP16</td>
<td>Intramuscular injections</td>
<td>Vehicle</td>
<td>300</td>
<td>PWT, 6 months</td>
<td>Ongoing</td>
<td><a href="http://www.clinicaltrials.gov">www.clinicaltrials.gov</a> (Unpublished)</td>
</tr>
</tbody>
</table>

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.

<sup>1</sup> 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

Adventitia
Media
Internal elastic lamina
Endothelium

Lumen

Uninjured artery

Balloon inflation to denude the endothelium

Complete loss of endothelium

Neointima

Partial re-endothelialisation

1 day post injury

14 days post injury

28 days post injury

Neointimal regression

Regenerated endothelium
KAT301‘Endocardial VEGF-D Gene Therapy for Severe Coronary Heart Disease’: rationale

- Preclinical evaluation of AdVEGF-D^{ΔNΔC} in the pig model of acute pig myocardial infarction (Lähteenvuo et al, 2013).

- NOGA-guided intramyocardial injection achieved efficient & localised transduction (>50% in 1 cm³)

- 4-fold increase in mean capillary area up to 21 days after gene transfer. 20% increase in ejection fraction in the AdVEGF-D^{ΔNΔC} treated pigs 21 days after gene transfer, as compared to the time of occlusion. Perfusion in the AdVEGF D^{ΔNΔC} group remained 2.8-fold higher at the infarction area 21 days after infarction.

- AdVEGF-D^{ΔNΔC} 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, pharmacokinetics

- 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

Therapeutic Angiogenesis

Benefits

- Symptomatic improvement

Concerns

- Vector-associated toxicity
- Abnormal vascular growth in non-target tissues
- Latent tumour growth?
- Pro-atherogenic?
Therapeutic angiogenesis – potential adverse effects

- Increased tumour burden


- Increase in plaque rupture & arteriothromboembolic events

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

<table>
<thead>
<tr>
<th>Growth factor / cytokine</th>
<th>Localization within plaque</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α,β3</td>
<td>Expression within macrophage foam cells</td>
<td>85</td>
</tr>
<tr>
<td>VEGF/VEGFR</td>
<td>VEGF-A, VEGF-B, VEGFR-1, and VEGFR-2 staining evident within plaque SMCs</td>
<td>37</td>
</tr>
<tr>
<td>FGF2</td>
<td>Secreted by intraplaque mast cells</td>
<td>86</td>
</tr>
<tr>
<td>PD-ECGF</td>
<td>Expression within plaque macrophages and ECs of plaque neo-vessels, from coronary atherectomy specimens.</td>
<td>87</td>
</tr>
<tr>
<td>PAF</td>
<td>Expression correlated with CD68+ve monocytes</td>
<td>88</td>
</tr>
<tr>
<td>PDGF-A and-B</td>
<td>Expression correlated with SMCs and macrophages</td>
<td>89</td>
</tr>
<tr>
<td>HGF</td>
<td>Expression correlated with carotid atherosclerotic plaques, but not normal arteries</td>
<td>90</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Expression with activated macrophages, T lymphocytes and SMCs</td>
<td>91</td>
</tr>
<tr>
<td>HB-EGF</td>
<td>Macrophages and SMCs</td>
<td>92</td>
</tr>
<tr>
<td>IL-8</td>
<td>Protein and mRNA present within direct coronary atherectomy homogenates</td>
<td>49</td>
</tr>
<tr>
<td>tPA, uPA</td>
<td>Intimal SMCs, macrophage-derived foam cells and plaque neo-vessels</td>
<td>93</td>
</tr>
</tbody>
</table>

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

Surrogate end-points of cardiac regeneration therapies

Perfusion
- SPECT
  - TI-201
  - Tc-99m PET
  - NH3 Contrast echo cMRI

Function
- Echocardiography cMRI
- Contrast ventriculogr. MUGA

Viability
- SPECT
  - TI-201 PET
  - 18FDG NOGA mapping
    - UPV map
    - Stress echo cMRI
    - Late enhancement
    - transmurality

Angiogenesis
- SPECT
  - Tc-Maracilatide
  - 123 I-VEGF* PET
  - 18FDG*
  - 124 I-HuMV833*
  - CT
  - CEU cMRI

## Comparison of different types of neovascularization

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


**Stem cell therapy for Cardiovascular Disease**


## Apparent Janus-Like Effects of Cells on Atherogenesis and Collaterogenesis

<table>
<thead>
<tr>
<th></th>
<th>Atherogenesis</th>
<th>Collaterogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-derived MNC</td>
<td>Increases(^{70}), inhibits(^{71})</td>
<td>Increases(^{65-69})</td>
</tr>
<tr>
<td>Monocyte/macrophage</td>
<td>Increases(^{10,23,53})</td>
<td>Increases(^{10})</td>
</tr>
<tr>
<td>T cells</td>
<td>Increases(^{23,53,72,73})</td>
<td>Increases(^{28})</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Increases(^{53,74})</td>
<td>Increases(^{75})</td>
</tr>
</tbody>
</table>

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


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 myocardium

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

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

*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)