

Basic science for the clinician

Angiogenic gene therapy in cardiovascular diseases: dream or vision?

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Chronic cardiovascular diseases are significant health problems. Although current treatment strategies have tremendously improved disease management, up to 30% of these patients cannot be successfully treated with current treatment approaches and new treatment strategies are clearly needed. Gene therapy and therapeutic vascular growth may provide a new treatment option for these patients. Several growth factors, like vascular endothelial growth factors, fibroblast growth factors and hepatocyte growth factor have been tested in clinical trials. However, apart from demonstration of increased vascularity, very few results with clinical significance have been obtained. Problems with gene transfer efficiency, short duration of transgene expression, selection of endpoints, and suboptimal patients for gene therapy have been recognized. Ongoing gene therapy trials have included improvements in study protocols, vector delivery and endpoints, addressing the identified problems. Better, targeted delivery systems and new, more optimal growth factors have been taken to clinical testing. Recent advances in these areas will be discussed and the concept of angiogenic therapy as a sole treatment is re-evaluated. A combination with regenerative therapies or standard revascularization operations might be needed to improve tissue function and clinical benefits.

Keywords Gene therapy • Angiogenesis • Coronary heart disease • Peripheral arterial disease • Heart failure • Gene delivery • Growth factors

Introduction

Cardiovascular diseases (CVD) represent a major healthcare problem throughout the world and continue to be the leading cause of mortality and morbidity worldwide.^{1,2} Additionally, many patients are not suitable for current treatments due to e.g. diffuse chronic disease or comorbidities. Thus, there is a clear need to develop novel treatment options that would have an impact not only on the symptoms of the disease but would address the underlying pathological processes.

Therapeutic angiogenesis offers a potential approach for improving ischemic tissue function by stimulating blood vessel growth, increasing tissue perfusion and supporting tissue regeneration and recovery.^{3,4} The potential advantages of local gene-based therapy include: (i) possibility for sustained long-term exposure to therapeutic compounds, (ii) ability to target therapies to specific cell types or tissues,

and (iii) decreased risk of systemic side-effects as compared to regular pharmacotherapy. Many angiogenic growth factors and transcription factors have been identified (see⁵ for review) which also possess other functions related to e.g. cell cycle, proliferation, energy metabolism and survival (Figure 1).

However, the local concentration of angiogenic factors is highly dependent on the used vector system and delivery route.⁶ Gene delivery approaches can be divided into direct tissue injections and intravascular infusions with or without surgical or catheter-mediated interventions or tissue permeabilization treatments (Figure 2). Gene transfer vectors, carrying the transgene, are usually either of plasmid or viral origin and vary in efficiency and duration of gene expression and immunogenicity (Figure 2) (see^{5,7} for review). Optimization of these factors should improve possibilities to achieve clinically significant results.

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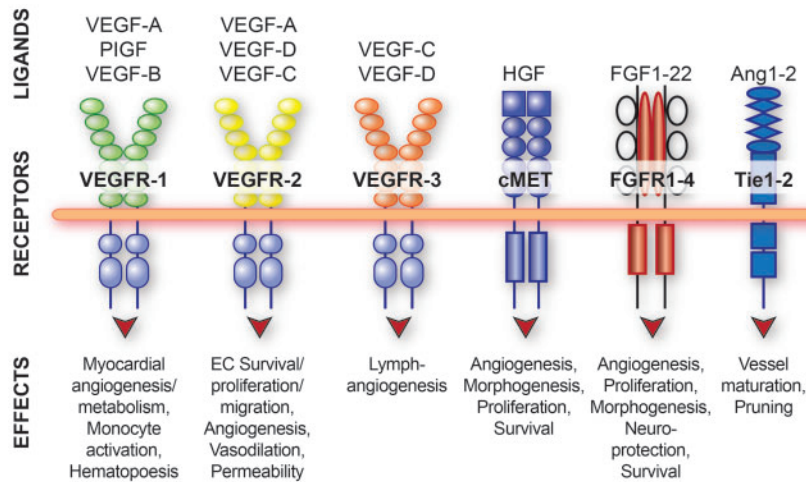


Figure 1 Growth factor and receptor—families related to blood vessel growth as well as their other functions.

Evaluation of clinical angiogenic gene therapy trials

Ischemic heart disease

Failure to perform complete myocardial revascularization is associated with decreased survival and recurrent angina. Current management strategies for these patients are limited and so-called refractory angina patients make up to 5% of patients in cardiology clinics.^{7–9} In the past randomized controlled trials (RCTs) such as Euroinject One,¹⁰ KAT¹¹, and NORTHERN¹² have tested percutaneous intramyocardial delivery of either naked plasmid or adenoviral vascular endothelial growth factor (VEGF)—A in coronary artery disease (CAD). VEGF-A gene therapy has also been combined with cardiac bypass grafting and even performed using epicardial intramyocardial injections via minithoracotomy as a sole therapy.¹³ However, the results have not been very promising (Supplementary material online, Table S1) except for safety of which over 10 years follow-ups have been conducted with no significant transgene or vector related side effects.^{14–16}

Currently, there are four angiogenic gene therapy trials ongoing in CAD (Table 1). Two trials are testing VEGFs for the treatment of myocardial ischemia. KAT301-trial tests a novel VEGF-D, in 30 patients with refractory angina and no revascularization opportunities (Table 1). This phase I/IIa RCT uses increasing doses of endocardial Ad-injections with electroanatomical targeting of injections using a NOGA[®] catheter system. Absolute myocardial blood flow will be measured using¹⁵ O-radiowater-PET at 3 and 12 months after the therapy. VEGF-D has not been tested in humans before and it stimulates both angiogenesis and lymphangiogenesis, thus representing a new therapeutic vascular growth strategy to treat refractory angina.¹⁷ A trial with adenovirus expressing all three major isoforms of human VEGF-A (VEGF-A116A)¹⁸ is also planned using intramyocardial injection via thoracotomy (Table 1).

Of other angiogenic factors, intracoronary adenoviral fibroblast growth factor (FGF)-4 has shown significant improvements in post-

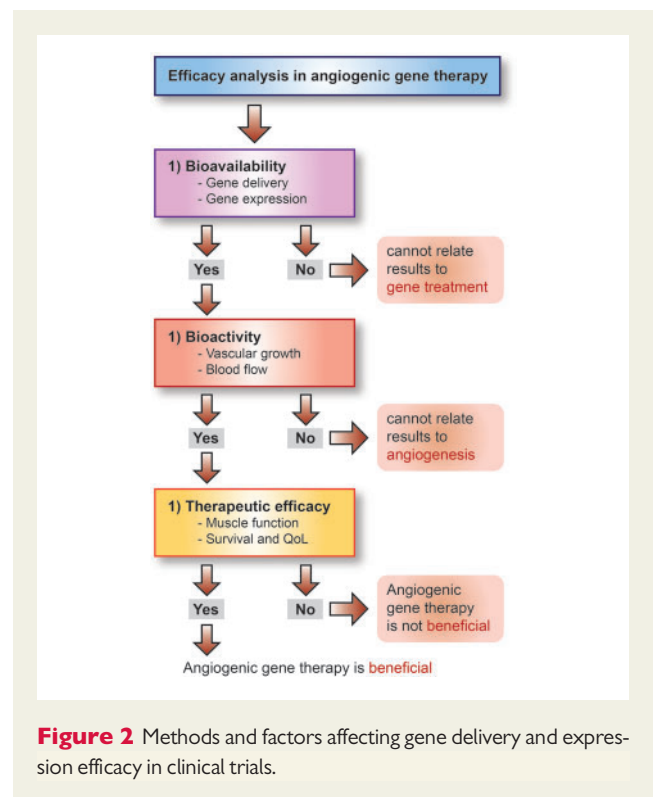


Figure 2 Methods and factors affecting gene delivery and expression efficacy in clinical trials.

menopausal women in a series of AGENT-trials.¹⁹ In a currently recruiting ASPIRE-trial (Table 1), intracoronary AdFGF-4 will be compared to standard care without a placebo group in an open-labelled design in 100 patients.²⁰ The primary endpoint is the change in reversible perfusion defect size using SPECT imaging at 8 weeks. A randomized, double-blinded, placebo-controlled AWARE-trial is also planned to test intracoronary AdFGF-4 only in women with stable

Table 1 Currently on-going or planned gene therapy trials in coronary artery disease and peripheral arterial disease

Trial name or ID	Disease	GP	Vector	Delivery	Pat no	Novelty	Status
Trials aiming for physiological angiogenesis							
NCT01757223	CAD	VEGF-A116A	Ad	i.my.(tct)	41	Expression of 3 different VEGF-A isoforms	Planned
NCT00956332	PAD	VEGF-A+Ang-1	RV	i.a.	28	Ang-1 should stabilize vessels induced by VEGF-A +RV used	Results pending
NCT00390767	PAD	VEGF-A+Ang-1	RV	i.a.	12	Ang-1 should stabilize vessels induced by VEGF-A +RV used	Results pending
Trials using therapeutic vascular growth in combination with other treatments							
KAT-PAD101 EudraCT2012-001019-22	PAD	VEGF-DdNdC	Ad	i.m.	30	Gene transfer 1-2 days before operation to improve distal runoff from surgical bypass graft in PAD	Recruiting
Trials using therapeutic vascular growth with aim for reduced side-effects							
KAT301 EudraCT2008-003295-22	CAD	VEGF-DdNdC	Ad	i.my.(cat)	30	Stimulation of both angiogenesis and lymphangiogenesis to improve cardiac fluid removal and decrease edema	Results pending
Trials with mitogenic and multifunctional growth factors							
ASPIRE	CAD	FGF-4	Ad	i.c.(cat)	100	Phase III study, 3x dosage compared to previous AGENT-2 trial	Recruiting
AWARE	CAD	FGF-4	Ad	i.c. (cat)	300	only women recruited	Planned
NCT02276937	PAD	FGF-2	SeV	i.m.	60	SeV used	Recruiting
NCT01548378	PAD	HGF	Pl	i.m.	200	A large HGF trial	Results pending
NCT02144610	PAD	HGF	Pl	i.m.	500	A large HGF trial	Recruiting

Ad, adenovirus; cat, catheter-mediated; GP, gene product; i.a., intra-arterial; i.c., intracoronary; i.m., intramuscular; i.my.: intramyocardial; Pl, plasmid; RV, retrovirus; SeV, sendai-virus; tct, thoracotomy.

angina. However, the recruitment has not yet started (Table 1). Hepatocyte growth factor (HGF) either in plasmid or adenoviral constructs has been tested in CAD but to date only in small open-label studies.^{21,22} As the placebo effect is strong in angiogenic therapies,^{4,23} the RCT-study design is essential for testing efficacy (Figure 3). Additionally, hypoxia inducible factor-1 alpha (Hif-1a) has been combined with coronary bypass grafting in a placebo-controlled study with 13 patients²⁴ but larger studies would be needed to support efficacy.

Heart failure

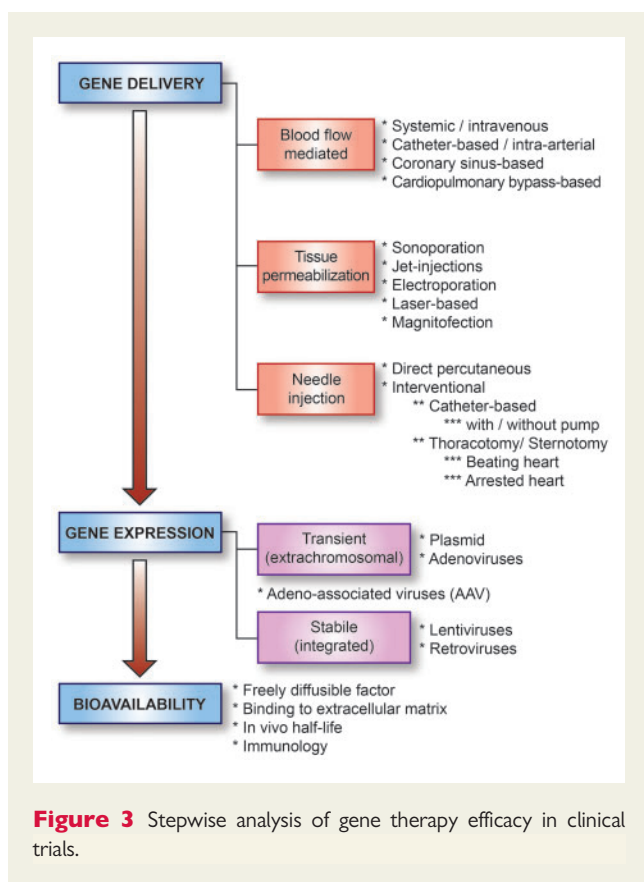
Despite the fact that CAD is the most common cause of heart failure (HF), gene therapy targets for the treatment of these two diseases differ. Gene therapy in HF has been focused primarily on excitation-contraction coupling and reduction of adverse remodelling regardless of aetiology. Recent large RCTs have used intracoronary injections of adeno-associated virus (AAV)-mediated SERCA2a²⁵ affecting myocardial calcium handling and transendocardial plasmid stromal cell derived factor (SDF)-1 mobilizing stem cells²⁶ but the results have been disappointing. Although inefficient gene expression probably explains the negative findings in the past HF trials,²⁷ a combination with short term angiogenic therapies could also be considered to balance cardiac metabolism and microcirculatory blood flow. For example, initially angiogenic VEGF-B has been shown to also activate expression of genes involved in the regulation of myocardial contractility and metabolism as well as to protect cardiomyocytes from

apoptosis and ischemic damage through physiological hypertrophy.^{28,29} However, the timing and duration of VEGF-B expression seem essential as too long VEGF-B expression can controversially decrease survival.²⁸

Peripheral arterial disease

Peripheral arterial disease (PAD) affects approximately 200 million people worldwide and its prevalence is increasing.³⁰ Current pharmacological treatments are not effective in PAD and not all patients are suitable for operational treatments.³¹ Locally administrated angiogenic gene therapies have been repeatedly safe and feasible also for severely diseased patients with no other suitable treatment options^{4,16} (Supplementary material online, Table S1). Even though a plasmid encoding VEGF-A165 'Neovasculgen',³² has been approved for clinical use for the treatment of PAD in Russia, a meta-analysis of RCTs with nearly 1500 treated patients found no consistent benefits of angiogenic gene therapy in PAD.³³

RCTs have been able to show some vascular growth effects of both plasmid and Ad-mediated VEGF-A gene therapy in PAD patients but have not found a consistent association of the neovascularity with functional parameters³⁴⁻³⁶ (Supplementary material online, Table S1). Leaky non-stable vessels have been blamed for the lack of functional benefits and current efforts have been directed towards improving functionality of the induced neovessels e.g. using a combination of VEGF-A and Angiopoetin (Ang)-1 (Table 1). However, two previous RCTs with plasmid Del-1 (DELTA-1-trial³⁷)



and adenoviral Hif-1 α /VP16 (WALK-trial³⁸) aiming for more physiological angiogenesis through effects on angiogenic transcription pathways found no beneficial effects on function. Functional improvements were neither found with FGF-1 plasmid in large RCTs TALISMAN³⁹ and TAMARIS.²³ A currently on-going trial is testing Sendai virus⁴⁰ mediated FGF-2 in PAD (Table 1). As a novel approach for PAD, a combination with surgical revascularization is tested in an ongoing KAT-PAD101-trial with intramuscular adenoviral VEGF-D given 1–2 days prior to vascular surgery to improve distal run-off after the operation (Table 1).

Although clinical trials in PAD patients have largely been unsuccessful, there are some auspicious signs. Significantly increased tissue oxygenation as well as improvements in peak walking time, ulcer healing and reduced amputation rates have been reported by several RCTs using HGF^{41–43} (Supplementary material online, Table S1). A current RCT is studying the effects of a NL003CLI-II-plasmid expressing two isoforms of HGF on ulcer healing in 200-patients (Table 1).

Key points learned from clinical trials

Patient selection

The meta-analysis of PAD trials showed no difference in the trial results according to disease severity (claudication vs critical

ischemia).³³ However, the patients may also differ in their comorbidities, such as hypertension or hypercholesterolemia as well as ongoing pharmacological treatments which could have unpredictable effects on vascular growth. Thus, in future trials, patient groups should be better categorized and patient cohorts of responders and non-responders should be studied in more detail to identify factors and biomarkers affecting treatment responsiveness. Knowledge of endogenous levels of vascular growth-associated factors might also be valuable background information.⁴⁴

Gene delivery

A delivery method which provides effective gene transfer with the capability of global transduction of the treated organ with minimal off-target gene expression would be desired. Intravascular delivery may be limited because of multiple arterial occlusions preventing delivery of the gene to the ischemic areas. At the same time, a considerable proportion of the therapeutic construct usually enters systemic circulation being exposed to the immune system and affecting non-target tissues. Although highly important for targeted gene delivery into the myocardium,^{10,45} catheter-based approaches even with electromechanical mapping are less specific than direct intramuscular injections to lower limb muscles or intramyocardial delivery during cardiac surgery with an arrested heart, providing better control for the procedure.

Vectors

Regarding its significance, it is surprising how few clinical trials have actually tried to demonstrate adequate gene transfer efficiency in human target tissues.^{46,47} To achieve consistent positive results, vector development would be essential in targeting gene expression only to treated tissues, escaping the immune system and maintaining long enough transgene expression for biological effects to take place.^{48,49} Clinically often used plasmids have low immunogenicity but suffer from low transduction efficacy. Adenoviruses cause strong but transient gene expression that may be limited by neutralizing antibodies in some individuals. For longer and more stable gene expression, AAVs and genome-integrating lentiviruses may be considered with AAVs offering also serotype-specific tissue targeting.⁵ However, it is still debated what is sufficient and long enough gene expression for each particular indication. Past clinical trials have not clearly documented whether the lack of therapeutic effects has been due to the low gene transfer efficiency, insufficient biological availability or low bioactivity of the gene product (Figure 3). Thus, vectors allowing monitoring and regulation of the transgene expression in the target tissues should be developed.

Endpoints

Clinical trial design with appropriate endpoints is of critical importance. Claiming therapeutic effects without demonstration of the presence of the therapeutic protein should be avoided (Figure 3). It would be essential to design and validate surrogate markers for both transduction efficacy and biological activity of the gene product. Objective surrogate endpoint measurements, such as PET, MRI, ECG and ultrasound should be favoured instead of subjective or unspecific measures, such as exercise testing or quality of life questionnaires. Also, the sensitivity of the methods employed should be re-evaluated as for example SPECT is not able to detect angiogenesis in the

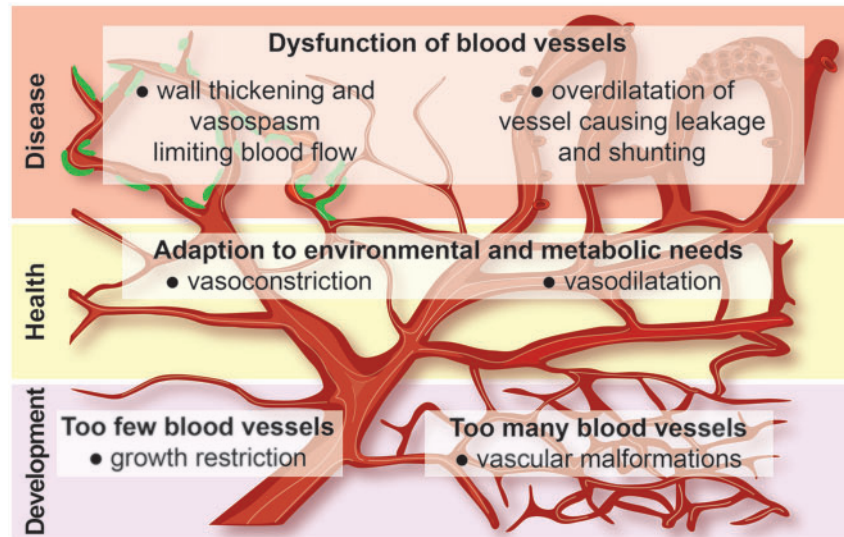


Figure 4 The dynamic nature of the vasculature in health, disease and development.

capillary level vessels. It may also be questioned whether endpoints like overall mortality, can reliably capture potentially significant treatment effects, such as number of hospital admissions or additional interventions in chronically ill aged individuals.

General conclusions and visions for the future

On the basis of the clinical trials in angiogenic gene therapy, there seems to be a clear disconnect between promising preclinical results and disappointments in RCTs.^{5,33,44} Signs of improved vascularity have been detected after angiogenic gene delivery in some RCTs suggesting efficient gene delivery and biological activity of the transgenes^{11,34} but translation to functional benefits in the patients lacks convincing evidence (Supplementary material online, *Table S1*). A re-evaluation is thus needed.

While development of gene delivery vectors is still needed to improve gene transfer efficacy and gene expression in human tissues, the concept of therapeutic vascular growth in the treatment of CVD is evolving. Affecting mainly the capillary level, angiogenic gene therapy might not be sufficient alone to promote revascularization in the scale of a human as compared to a laboratory mouse.^{6,44} Instead, it might locally enhance distal run-off after surgical or radiological revascularization procedures or improve outcome of incomplete revascularization. Induction of lymphatic growth, to reduce revascularization- and angiogenesis-related oedema, might also be beneficial.⁵⁰

Additionally, as the recovery potential in animal models may differ significantly from that in the human patients,⁴⁴ the enhanced capillary level vascularity might not be enough to improve recovery of the severely affected and even necrotic human tissues. The mitogenic and myogenic properties of e.g. HGF working besides angiogenesis

might help to restore function and could explain some positive clinical results. Synergy with regenerative gene or cell therapies should also be considered.

It may also be argued that if tissue ischemia is not cured with angiogenesis, maybe the clinical problem is then not the lack of angiogenesis. Hypoxia initiated vascular growth takes place already in the very early phases of embryonal development and continues its dynamic role as part of the body's physiological and pathological responses, e.g. in wound healing, throughout life. The dysfunction of blood vessels that is related to different disease conditions (*Figure 4*) may still possess many unidentified features that could help us to develop novel therapies for CVD. But for establishment of new treatments, preclinical models need to more accurately resemble the chronic nature of human diseases in order to better evaluate the efficacy of novel treatments in humans.⁴⁴

Key points

- Comorbidities and pharmacological treatments might have unpredictable effects on vascular growth. → Patient cohorts of *responders and non-responders* should be studied to identify those benefitting from the treatments.
- Gene transfer should yield high transduction efficiency in the target tissue without off-target side-effects. → *Targeted and regulated vectors* need to be developed.
- Optimal duration and level of gene expression in angiogenic therapies has not yet been established. → *Biological availability* as well as *bioactivity* of the gene product need to be studied in more detail in future trials.

- Subjective measures, such as exercise testing, may suffer from a significant placebo effect. → Objective surrogate markers need to be developed and validated to measure treatment effects.
- Angiogenic gene therapy alone might not be sufficient to promote revascularization in large muscle areas. → Combination with established surgical or catheter-based revascularization procedures should be evaluated. Also, the growth of the whole vascular tree including lymphatics (i.e. therapeutic vascular growth) should be explored.
- Increasing capillary level vascularity might not be enough to enhance recovery in severely affected and even necrotic tissues. → Multifunctional growth factors and combination with regenerative therapies should be evaluated.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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