Cardiovascular gene therapy: past, present, and future

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Cardiovascular gene therapy
Past, Present and Future

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Abstract

Cardiovascular diseases remain a large global health problem. While several conventional small molecule treatments are available for common cardiovascular problems, gene therapy is a potential treatment option for acquired and inherited cardiovascular diseases that remain with unmet clinical need. Among potential targets for gene therapy are severe cardiac and peripheral ischemia, heart failure, vein graft failure and some forms of dyslipidemias. The first approved gene therapy in Western world was indicated for lipoprotein lipase deficiency, which causes high plasma triglyceride levels. With improved gene delivery methods and more efficient vectors together with interventional transgene strategies aligned to a better understanding of the pathophysiology of these diseases, new approaches are currently tested for safety and efficacy in clinical trials. In this article we integrate an historical perspective with recent advances that will likely impact on clinical development in this research area.

Key words:

cardiovascular gene therapy, clinical trials, hyperlipidemias, ischemia, heart failure
Introduction

Potential of cardiovascular gene therapy became evident in the late 1980s when it was shown that direct intraarterial gene transfer was possible with endovascular catheter techniques (1). At the same time hyperlipidemias became a target for gene therapy (2) and topics like in-stent restenosis, vein graft stenosis, heart failure, arrhythmias, refractory angina and peripheral vascular disease were recognized as potential targets for gene therapy. In the clinics, pioneering work of Jeff Isner and colleagues used plasmid gene transfer to treat severe peripheral vascular disease (3) and adenoviral vectors were for the first time used for local endovascular catheter-mediated gene therapy in humans (4).

In spite of great enthusiasm and positive preclinical results, clinical translation of cardiovascular gene therapy has not yet been very successful. There are many factors that might contribute to this negativity, including insufficient gene delivery to the target site undermining the potential of transgenes, too short duration of transgene expression from particular transduction strategies, insufficient knowledge about underlying pathophysiological mechanisms leading to retrospectively poor strategy and often underpowered clinical trial design. Several prominent trials that have produced negative results and failures have sent investigators back to the laboratory in order to re-evaluate and design better vectors and gene therapy approaches for cardiovascular diseases (5,6). This bench-bedside iteration has been fruitful in other gene therapy arenas, but has thus far failed to have significant impact in the cardiovascular space.

In spite of these difficulties, during the last 2-3 years, significant clinical and conceptual progress has been made in cardiovascular gene therapy. The first gene drug approved in Western world, Glybera, is indicated for the treatment of severe lipoprotein lipase deficiency. Even though this condition is ultra rare, it was an important milestone for the entire field of gene therapy (7). Additionally, gene delivery techniques have been significantly improved, particularly with respect to catheter-based approaches and targeting of powerful new therapeutic genes to myocardium has recently produced promising results (8) Thus, a new generation of cardiovascular clinical trials is primed to evaluate the potential of gene therapy in carefully selected patient populations. This wave of trials must include carefully documented gene transfer effects and objectively measurable changes in parameters like blood flow, metabolic activity and cardiac function (9) as the impact of these results cannot be underestimated. Potential targets for cardiovascular gene therapy are presented in Figure 1.

Coronary heart disease

Coronary heart disease is caused by insufficient blood flow to myocardium due to atherosclerotic stenoses in the main coronary arteries. It is well known that some patients can develop collateral arteries which rescue myocardium in spite of the stenoses. Therefore, it is logical that this condition could be treated by providing new blood flow to the ischemic muscle (10). Coronary bypass surgery and angioplasty techniques are excellent examples of this treatment principle. However, these approaches are not possible for all patients and there is an
increasing group of severe angina pectoris patients, so-called refractory angina, who cannot be treated with these conventional approaches (11).

Since basic mechanisms of angiogenesis and blood vessel formation are already well-known, therapeutic vascular growth, which tries to grow new blood vessels in the ischemic myocardium, is a potential new treatment option. Several growth factors have been used to achieve this goal in the past, but until recently, no clinically significant results have been obtained in phase II/III clinical trials (6). The most promising candidates for therapeutic vascular growth seem to be members of the VEGF and FGF families, HGF and gene therapy approaches combined with cell therapy (12,13).

During the last decade several randomized controlled trials have tested either naked plasmids or adenoviral vectors for the treatment of severe coronary heart disease. Trials like EuroinjectOne (14), KAT (15), REVASC (16), NOTHERN (17), NOVA (18), VEGF-Neupogen (19) and GENASIS (20) have tested intramyocardial delivery of VEGFs (Table 1). Gene transfer has also been used during bypass surgery and via minithoracotomy using epicardial injections (21,22). Importantly, safety in these trials has been very good even after ten years follow-up (23-25). At the moment there are five angiogenic gene therapy trials that are either ongoing or have recently reported final results (Table 1). Two of these trials are testing a novel VEGF-D\textsuperscript{D\textsc{Nlc}} in refractory angina patients using percutaneous endocardial delivery of adenoviral vectors (8). Targeting to ischemic hibernating myocardium is done using a combination of electroanatomical mapping and $^{15}$O-$\text{H}_2\text{O}$-PET which makes it possible to treat areas which suffer from stress-induced ischemia (9). Crucially, absolute myocardial blood flow can be measured with $^{15}$O-$\text{H}_2\text{O}$-PET and correlated to potential clinical benefits. Since VEGF-D\textsuperscript{D\textsc{Nlc}} is both angiogenic and lymphangiogenic growth factor, it represents a new strategy (i.e. therapeutic vascular growth) to treat refractory angina in addition to angiogenic effects: stimulation on lymphatic circulation will help to reduce myocardial edema which is a common side effect after proangiogenic therapies (14-18). VEGF-D\textsuperscript{D\textsc{Nlc}} can also potentially induce regenerative effects by inducing stem and progenitor cells in the treated muscles (26). A new trial based on intramyocardial injections via thoracotomy has also been planned. This study is based on adenovirus expressing three major VEGF-A isoforms which should lead to a better angiogenic response by generating more natural growth factor gradients between ischemic and normoxic parts of the myocardium. (27).

Adenoviral intracoronary delivery of FGF-4 has been thoroughly tested in a series of AGENT-trials (28). Currently, the ASPIRE-trial will compare standard of care without a placebo group in an open label design with primary endpoint based on relative perfusion changes in the myocardium as measured with $^{99}$Tc-SPECT imaging at 8 weeks (29). The randomized double-blinded placebo-controlled AWARE-trial has also been planned with intracoronary AdFGF-4 in women with stable angina (Table 1). Both ASPIRE and AWARE trials are based on significant positive effects on exercise tolerance test found in AGENT 3 and 4 trials in > 55 yr women (28). HGF has been used both as plasmid and adenoviral constructs in the treatment of coronary heart disease but so far only small open-label studies have been reported (30,31).
Potential new treatments for refractory angina include upregulation of hypoxia inducible factor-1α, thymosin B4, modified stabilized RNAs, GalNac-antisense oligonucleotides, nanoparticles, promoter-activating siRNAs and exosomes which could deliver both growth factors and miRs to ischemic myocardium (6, 32-38). No trials are currently testing these new approaches for the treatment of severe coronary heart disease. However, thymosin B4 is an interesting candidate since it activates partly different signaling cascades than previously used growth factors (34). Also, epicardial activation of cardiomyocyte proliferation and tissue repair could prevent harmful remodeling and be a useful approach for therapy (35).

Peripheral vascular disease

Peripheral vascular disease is caused by atherosclerotic occlusions in the main lower extremity arteries producing symptoms like claudication, rest pain and insufficient ulcer healing. Patients are usually older than those with coronary symptoms which makes this population very difficult to treat. Current pharmacological treatments are not very effective for critical limb ischemia or severe peripheral arterial disease and not all patients are suitable for endovascular operations. Therapeutic vascular growth with the same factors as used in coronary heart disease gene therapy have been tested with both intraarterial and intramuscular delivery routes. However, in a recent metaanalysis of randomized controlled trials no consistent benefits were found of angiogenic gene therapy in peripheral vascular disease (39). This illustrates the challenges ahead for this population, but the driver for innovation is the large unmet clinical need. Some positive reports are available from both plasmid and adenovirus-mediated VEGF-A gene therapy (40). Also, improvements in oxygenation as well as walking time and ulcer healing have been reported (41,42) and a plasmid-based VEGF-A product has been approved for clinical use in peripheral arterial disease in Russia (43). Two large FGF-1 plasmid trials TALISMAN (44) and TAMARIS (45) did not find functional improvements in the treated patients although TALISMAN trial showed a beneficial effect on the amputation rate in critical ischemia patients. FGF-2 is currently tested in PAD using a sendai virus vector but results have not been reported (46). Of the other large previous trials plasmid Del-1 (Delta-1 trial) (47) and adenoviral HIF-1α/vp16 (WALK trial) (48) aiming to stimulate angiogenic signaling pathways gave negative results.

Problems like long atherosclerotic stenoses in the main arteries, shunting and a stealing effect of the increased blood flow to less ischemic areas, unstable neovessels and too short time of transgene expression have been listed as potential reasons for failures (49). It appears that some basic physiological aspects of the peripheral muscle perfusion have not been fully taken into account when designing previous trials and it is likely that factors like overdilated capillaries, alterations in capillary blood transit time and insufficient transfer of oxygen into peripheral muscles need to be taken into account when designing future clinical trials (6,50). A recent trial aimed to improve functionality of the induced neovessels by using a combination of retroviral VEGF-A and angiopoietin-1 gene transfers to endothelial and smooth muscle cells isolated from patients with peripheral vascular disease, whereafter the cells were injected back to the patients (51). A combination with surgical revascularization is tested in an ongoing KAT-PAD101 trial where AdVEGF-D^{IndoC} is given 1-2 days prior to vascular surgery in order to dilate capillaries and
small arterioles to improve distal runoff after the vascular surgery operation (52). In addition, AdVEGF-D^\text{NdC} should induce both angiogenic and lymphangiogenic growth in the treated legs (Table 1). Two trials have tested HGF in critical limb ischemia (53,54) and a current trial is ongoing using a plasmid which expresses two HGF isoforms. Multiple intramuscular injections at several time points are used for gene delivery. SDF-1 plasmid is also tested in a similar set-up for critical limb ischemia (Table 1).

It appears that in the future critical limb ischemia patients should be studied separately from less severe claudicants and that more emphasis should be given to accurate imaging, metabolic and functional studies as surrogates for the treatment effect since walking distance, amputation rate or ulcer healing may not be able to capture improvements in the frequency of hospital admissions or related variables in these patient populations (55). It is possible that only a subgroup of peripheral vascular disease patients will benefit from therapeutic vascular growth while others may actually have problems other than insufficient angiogenesis (6). For future clinical trials it would be important to identify patient subgroups which are most likely to respond positively to the new treatments.

**Heart failure and arrhythmias**

Heart failure is an increasingly common problem in elderly population due to improved therapies for coronary heart disease and acute myocardial infarction. Advances in understanding of the pathogenesis of heart failure have led to recent gene therapy trials (Table 2) (5). CUPID2-trial tested AAV1-SERCA2a (sarcoplasmic reticulum calcium ATPase 2a) in patients with chronic systolic heart failure or non-ischemic cardiomyopathy (56). Previous smaller trials had indicated positive effects and therefore it was a great surprise that all endpoints in CUPID2-trial were negative. AAV1 vector was given with one-time intracoronary infusion but apparently, gene transfer efficacy remained too low to induce any measurable positive effects (57). AGENT-HF and SERCA-LVAD trials will test the same AAV1-SERCA2a in congestive heart failure and in patients with left ventricle assist device (LVAD), respectively (Table 2).

STOP-HF trial tested SDF-1 plasmid in heart failure. Therapy was given as several endomyocardial injections using a catheter device. Six minute walking distance was used as the primary endpoint but also this trial was negative (58). It appears that gene transfer efficiency was too low to achieve measurable positive outcomes. RETRO-HF trial will test a similar SDF-1 plasmid using a retrograde delivery to the heart muscle. Adenoviral adenylyl cyclase 6 will be tested in congestive heart failure patients using a dose escalation format. Primary endpoint will include exercise tolerance test and cardiac function measurements. However, results of these trials are not yet available (Table 2). An obvious concern for the adenylyl cyclase trial is the short expression time of adenovirus and potential immunological reactions, especially if repeated dosing will be required.

It is interesting to note that even though heart failure is caused most commonly by advanced coronary heart disease, treatment genes for heart failure have been very different from those used for coronary heart disease and myocardial ischemia, targeting primarily excitation-contraction coupling and adverse remodelling. In the future, combination with short-term angiogenic therapies could help to balance altered cardiac metabolism and microcirculatory
blood flow in failing myocardium. VEGF-B has shown several useful effects, such as cardiac-specific angiogenic activity and improvement in cardiac energy metabolism (59-62) which could be tested in clinical trials. Combined gene and cell therapies could also potentially be used in future trials to achieve positive treatment outcomes (12).

Heart failure develops gradually and while in the early phase targeting myocardial force, contractility and adverse remodelling are useful for therapy, in the later stages apoptosis of cardiomyocytes, fibrosis and arrhythmias bring significant challenges for the late state therapies. Since heart failure requires treatment of the majority of ventricular cardiomyocytes, very efficient vectors and gene delivery techniques are required for these indications. AAVs have shown significant long-term efficacy in preclinical models but this still needs to be proven clinically. Also, high prevalence of pre-existing antibodies against many AAV serotypes remains a concern. Recently, transfer of nucleic acids with slowly degrading nanoparticles and exosomes have become potential therapeutic approaches and new treatment genes, such as S100A1 and vectors with better cardiac tropism are being developed for heart failure therapy (36-38,63,64). The best scenario would obviously be an efficient early treatment of myocardial infarction so that remodeling and progression to heart failure could be prevented. Improving blood flow to salvageable myocardium and evoking cardiomyocyte proliferation after acute myocardial infarction could be useful in this regard.

Arrhythmias are a significant cause for cardiac morbidity and mortality. Atrial fibrillation is a very common problem and can lead to significant clinical sequelae. The most severe ventricular arrhythmias and conduction system defects could theoretically be amenable to gene therapy. For example, a malfunctioning sino-atrial node could be replaced with locally induced new focus which could control cardiac rhythm. However, these approaches have turned out to be very difficult in vivo and so far no clinical tests have been conducted in this area.

**Hyperlipidemias, lipoprotein metabolism and atherosclerosis**

Gene therapy could potentially be used for the treatment of severe inherited and acquired disorders of lipoprotein metabolism. The first approved gene drug in Western world was Glybera which is an AAV1 vector expressing lipoprotein lipase. This treatment is indicated for severe lipoprotein lipase deficiency which causes high postbrandial plasma triglyceride levels and severe pancreatitis attacks. The treatment is given by multiple intramuscular injections (7,65,66) (Table 2).

Historically, homozygous familial hypercholesterolemia which is caused by a mutation in low density lipoprotein (LDL) receptor, was the first disorder where gene therapy was tested in the clinics (2). The first trial was very laborious with a large liver resection and harvesting of hepatocytes, followed by retrovirus-mediated ex vivo gene transfer of LDL receptor and returning of the cells back to the patients. Results were not very convincing although a few patients showed a moderate reduction in plasma cholesterol level. Currently, a clinical trial is planned with AAV vector expressing LDL receptor to treat homozygous familial hypercholesterolemia (Table 2). Based on good clinical experiences with AAV vectors in the
treatment of haemophilia this approach sounds promising although potential immune responses against AAV vectors remain a concern.

Promising results have been obtained with stabilized, liver targeted antisense oligonucleotides against lipoprotein (Lp)a. Lp(a) is an independent risk factor for coronary heart disease and subcutaneous injections of antisense oligonucleotides have been reported to be safe and very efficient in reducing atherogenic Lp(a) levels (67). Other targets to reduce atherogenic lipoproteins are PCSK9 (68) and apolipoprotein C-III (69) which are currently tested for human therapy based on either antisense oligonucleotides or RNAi technology (Table 2). Mipomersen is already an approved antisense drug to reduce apolipoprotein B-100 levels in familial hypercholesterolemia and in some other types of severe hypercholesterolemias (70). However, significant side effects have also been reported with Mipomersen and this drug is not very often used for therapy. Nevertheless, liver seems to be an excellent target for antisense and RNAi therapy for lipid and lipoprotein disorders.

Some dyslipidemias and even atherosclerosis could be treated by increasing apolipoprotein A-1 levels (71), since apoA-1 is a key component in antiatherogenic HDL particles. Lecithin cholesterol acyl transferase is a key enzyme in HDL maturation and a potential target for therapy. Also, inhibition of microsomal triglyceride transfer proteins has shown potential to control severe atherogenic lipoprotein profiles. Since oxidized LDL is a key pathogenetic factor for the accumulation of lipids in the arterial wall (72), soluble decoy scavenger receptors, which can bind oxidized LDL and sequester it for degradation in the liver (73) or vectors expressing antibodies against oxidized LDL (74) could theoretically be used as antiatherogenic therapy. However, it is clear that gene therapy approaches to control dyslipidemias and atherosclerosis can only be potentially used in very severe cases where well-characterized and effective conventional therapies, such as statins and inhibitors of cholesterol absorption, cannot achieve desired treatment effects.

**Restenosis, in-stent restenosis and vein graft disease**

Intravascular interventions to target occlusions in the coronary arteries inevitably cause marked damages to endothelium and arterial medial layer exposing thrombogenic molecules. Activation of platelets, thrombosis, leukocyte adhesion, smooth muscle cell proliferation, matrix accumulation and vascular remodeling are all involved in the pathogenesis of restenosis, i.e. the response to injury leading to re-narrowing of the vessel lumen (75). Drug-eluting stents have significantly improved treatment outcomes and reduced remodeling, recoil and acute obstruction of the treated arteries although late stent thrombosis still occurs in some patients. However, it is difficult to see that gene therapy could be competitive with current drug-eluting stents. Also, a previous clinical trial in restenosis gave negative results (76) (Table 2).

Vein graft stenosis is another common problem after bypass surgery. With most bypass operations still using the autologous saphenous vein as a conduit for bypass, there remains a significant risk of failure in the long term. The rationale for a gene therapy approach is to prevent pathological vein graft remodeling in the immediate time post implantation as the vein
adapts to higher blood pressure and surgical handling. Vein grafts offer an excellent opportunity for local intraluminal or perivascular gene transfer during the preparation of grafts ex vivo (77,78). TIMPs have been suggested as a potential treatment (77,78) but no clinical results are yet available from this approach, due to problems in manufacturing clinical grade AdTIMP-3. E2F transcription factor antisense decoys have been tried in clinics but the results were disappointing (79,80) (Table 2). Vascular grafts and dialysis anastomosis stenosis could also be treated using gene transfer from the adventitial surface of the vessels. Phase I/II studies have indicated that local transduction with VEGF could reduce stenosis, presumably by increasing nitric oxide and prostacyclin production in the grafts. However, final clinical trial results are not available from these studies (81).

Lessons learned and future perspectives

Gene delivery vectors and transduction efficiency

It appears that gene transfer efficiency in many previous cardiovascular trials has been too low to achieve meaningful clinical effects. Usually, in preclinical animal models much higher doses per kilogram of gene transfer vectors have been used than what is possible in human trials (82). Intraarterial delivery methods seem to be much less efficient than intramyocardial or intramuscular injections (4). As a consequence, it is likely that the concentration of therapeutic proteins in the target tissues has not reached sufficient levels and/or has not persisted long enough to achieve biological effects. Secreted therapeutic proteins have a better likelihood to give positive effects since currently it is very difficult to achieve more than 10-20% transduction efficiencies in human heart or peripheral muscles. Factors like volume per injection and matrix binding properties of the vectors and transgenes affect the distribution of transgene products in the treated tissues. It appears that physical (i.e. by surgery or via a catheter) or possibly genetic targeting of vectors would be desirable. In therapeutic vascular growth the expression time of transgene should be long enough to cause biological effects, but too long expression time of powerful factors like VEGF will be detrimental to the tissue architecture (83). In the future it would be important to develop vectors where transgene expression could be regulated by small molecules or physiological stimuli. Integrating vectors can best be used for applications where genetic defects require a lifelong treatment effect. It would be important to determine in preclinical studies and in phase 1 trials that transgene product can really be measured either in plasma or in the target tissues and that a dose effect could be demonstrated (84). Trouble shooting of the main problems in clinical gene therapy trials has been presented in Table 3.

Patient populations

In many previous trials severe “no-option” patients who are no longer eligible for any other kind of treatments have been recruited. However, in future ischemia and heart failure trials less severe patients should be recruited. It is possible that in the severe ischemia patients one of the main reasons for the difficult clinical situation is that endogenous angiogenesis has failed in the first place and that these patients are no longer able to react to angiogenic therapies. It is likely that only some subgroups of patients will respond positively to gene therapy approaches (8). It
would be highly desirable to find biomarkers or other characteristics that would help to select the most optimal patients for future clinical trials (6).

Placebo effect has been very strong in many previous cardiovascular gene therapy trials and only a randomized, blinded, controlled trial design can give reliable results regarding clinical benefits (82). Reasons for the strong placebo effect are partly unknown but patients usually go through heavy pre-evaluation screening process with multiple laboratory and imaging tests and gene therapy itself raises high expectations in severely affected patients. Therefore, it is not surprising that placebo groups have reacted positively. It should also be kept in mind that in intramuscular injections tissue injury caused by the needle or catheter can lead to local inflammation and production of growth factors and cause some positive effects. These factors should be taken into account when planning control groups for the future clinical trials (Table 3).

Endpoints

Whether technical or pharmacological shortcomings of gene drugs in the previous trials have caused clinical failures remains unknown. However, traditional endpoints, such as survival, exercise tolerance, amputation frequency or ulcer healing seem to be very demanding in severely affected patients (6). There is a clear need to develop validated surrogate endpoints for cardiovascular trials based on tissue perfusion, collateral flow, metabolic improvements and reduced burden of the use of hospital services which could better capture potential therapeutic benefits. This together with the selection of patients who are most likely to benefit from new therapies should be taken into account in the future clinical trials (6,8).

Evaluation of preexisting antibody levels and immunological responses to the treatments should be included in every trial and correlated with the potential treatment results. In future trials gene therapies should also be combined with existing therapies, such as bypass surgery or angioplasty as adjuvant therapies. This would allow new approaches for therapy, such as in peripheral vascular disease where gene transfer is given prior to bypass operation in order to open peripheral capillaries and to avoid a poor run-off syndrome which often leads to disappointing results in these patients (6,50,52). Other co-morbidities can significantly affect therapeutic outcomes and for this reason patients should be accurately stratified for diseases like diabetes, rheumatoid arthritis or other autoimmune diseases in order to avoid confounding results. Most therapeutic approaches have so far been based on single-dose applications. It should be evaluated whether repeated dosing could be applied since many chronic cardiovascular diseases are likely to progress and the need for future therapeutic interventions is quite obvious.

As far as safety profile of cardiovascular gene therapy is concerned, most trials have shown a very good safety profile even after ten year follow-up (23-25). This should encourage clinical testing of new therapeutic approaches with improved vectors and gene delivery methods. Current imaging methods can be used to guide therapeutic approaches so that very low doses could be applied to f.ex. ischemic areas of myocardium in combination with standard therapies. Since regulatory pathway for the approval of gene drugs has now been established, it is
expected that several novel treatments for cardiovascular diseases will enter clinical testing in
the near future.

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refractory angina patients. A phase I/IIa study with one year follow-up. Submitted.

Intramyocardial gene therapy directed to hibernating heart muscle using a combination of
electromechanical mapping and positron emission tomography. Hum Gene Ther. 27, 830-834.


Tables and Figure
| Trial         | Disease | Vector | Therapeutic agent | Delivery                        | Study design | n   | Primary endpoint                                                                 | Main result                                                                 | Reference          |
|--------------|---------|--------|-------------------|---------------------------------|--------------|-----|----------------------------------------------------------------------------------|                                                                            |                    |
| KAT301       | CAD     | Ad     | VEGF-D<sup>dirNc</sup> | Percutaneous i.my. NOGA/PET guided injections | Phase I, RCT | 30  | Safety, perfusion reserve in ischemic area (PET)                                  | Positive, increased perfusion reserve in treated areas at 1yr              | 8                  |
| ReGenHeart   | CAD     | Ad     | VEGF-D<sup>dirNc</sup> | Percutaneous i.my. NOGA/PET guided injections | Phase II, RCT | 180 | 6 min walking test, perfusion reserve in ischemic area (PET/SPECT)               | NA                                                             | NCT03039751        |
| ASPIRE       | CAD     | Ad     | FGF-4             | Percutaneous i.c. injections      | Phase III, open-label | 100 | Reversible perfusion defect (SPECT)                                               | NA                                                             | 29                 |
| AWARE        | CAD     | Ad     | FGF-4             | Percutaneous i.c. injections      | Phase III, open-label | 300 | Ischemic ECG changes on ETT                                                      | NA                                                             | NCT00438867        |
| VEGF-A116A   | CAD     | Ad     | VEGF-A<sub>116A</sub> | Thoracotomy i.my. injections      | Phase I/II, open-label | 41  | Time to 1 mm ST depression on ETT                                                 | NA                                                             | NCT01757223        |
| HGF-X7       | CAD     | Ad     | HGF               | Percutaneous i.my. injections     | Phase I, open-label, no controls | 12  | Safety                                                                           | NA                                                             | 30                 |
| MULTIGENE ANGIO | PAD | RV    | VEGF-A<sub>165, Ang1</sub> with cell therapy | Percutaneous i.a. injections | Phase I, open-label, no controls | 23  | Safety, amputation-free survival                                                  | Positive, amputation-free survival 72% at 1yr                           | 51                 |
| JVS-100      | PAD     | Pl     | SDF-1             | i.m. injection                   | Phase II      | 48  | Safety, amputation rate                                                           | NA                                                             | NCT01410331        |
| HGF-X7 (VM202) | PAD | Pl     | HGF               | i.m. injections                  | Phase II      | 50  | Visual analog scale                                                              | NA                                                             | NCT01064440        |
| HGF-X7 (NL003) | PAD | Pl     | HGF               | i.m. injections                  | Phase II, RCT | 200 | Ulcer area, visual analog scale                                                  | NA                                                             | NCT01548378        |
| KAT-PAD101   | PAD     | Ad     | VEGF-D<sup>dirNc</sup> | i.m. injections                  | Phase I, RCT  | 30  | Safety, perfusion in treated area (PET)                                          | NA                                                             | EudraCT 001019-22  |
| Neovasculgen | PAD     | Pl     | VEGF-A<sub>165</sub> | i.m. injections                  | Phase II/III, RCT | 100 | Pain-free walking distance                                                       | Positive, pain-free walking distance increased 167% at 1yr             | 43                 |
### Second part of Table 1

#### Previous trials in CAD and PAD

<table>
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<tr>
<th>Trial</th>
<th>Disease</th>
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<th>Therapeutic agent</th>
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<th>Study design</th>
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<td>CAD</td>
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<td>VEGF-Neupogen trial</td>
<td>CAD</td>
<td>Pl</td>
<td>VEGF-A\textsubscript{165}\textsubscript{r} rhGCSF</td>
<td>Percutaneous i.my. NOGA guided injections and systemic rhGCSF administration</td>
<td>Phase II, RCT</td>
<td>48</td>
<td>Change in myocardial perfusion (SPECT)</td>
<td>Negative</td>
<td>19</td>
</tr>
<tr>
<td>AGENT-3</td>
<td>CAD</td>
<td>Ad</td>
<td>FGF-4</td>
<td>Percutaneous i.c. injections</td>
<td>Phase III, RCT</td>
<td>416</td>
<td>ETT</td>
<td>Negative (subgroup of &gt; 55yr old women positive)</td>
<td>28</td>
</tr>
<tr>
<td>AGENT-4</td>
<td>CAD</td>
<td>Ad</td>
<td>FGF-4</td>
<td>Percutaneous i.c. injections</td>
<td>Phase III, RCT</td>
<td>116</td>
<td>ETT</td>
<td>Negative</td>
<td>28</td>
</tr>
</tbody>
</table>

### Third part of Table 1
<table>
<thead>
<tr>
<th>VEGF peripheral trial</th>
<th>PAD</th>
<th>Ad, Pl</th>
<th>VEGF-A&lt;sub&gt;165&lt;/sub&gt;</th>
<th>Percutaneous i.a. injection at angioplasty site</th>
<th>Phase II, RCT</th>
<th>54</th>
<th>Increased vascularity in angiography</th>
<th>Positive</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAVE</td>
<td>PAD</td>
<td>Ad</td>
<td>VEGF-A&lt;sub&gt;121&lt;/sub&gt;</td>
<td>i.m. injections</td>
<td>Phase II, RCT</td>
<td>105</td>
<td>Peak walking time</td>
<td>Negative</td>
<td>42</td>
</tr>
<tr>
<td>WALK</td>
<td>PAD</td>
<td>Ad</td>
<td>HIF-1α/VP16</td>
<td>i.m. injections</td>
<td>Phase III, RCT</td>
<td>289</td>
<td>Peak walking time</td>
<td>Negative</td>
<td>48</td>
</tr>
<tr>
<td>Delta-1</td>
<td>PAD</td>
<td>Pl</td>
<td>Del-1</td>
<td>i.m. injections</td>
<td>Phase II, RCT</td>
<td>105</td>
<td>Peak walking time</td>
<td>Negative</td>
<td>47</td>
</tr>
<tr>
<td>Groningen trial</td>
<td>PAD</td>
<td>Pl</td>
<td>VEGF-A&lt;sub&gt;165&lt;/sub&gt;</td>
<td>i.m. injections</td>
<td>Phase II, RCT</td>
<td>54</td>
<td>Decrease in amputation rate</td>
<td>Negative</td>
<td>41</td>
</tr>
<tr>
<td>HGF-STAT</td>
<td>PAD</td>
<td>Pl</td>
<td>HGF</td>
<td>i.m. injections</td>
<td>Phase II, RCT</td>
<td>104</td>
<td>Limb perfusion measured as TcPO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Positive, TcPO&lt;sub&gt;2&lt;/sub&gt; increased in high dose group at 6 mo</td>
<td>53</td>
</tr>
<tr>
<td>HGF study</td>
<td>PAD</td>
<td>Pl</td>
<td>HGF</td>
<td>i.m. injections</td>
<td>Phase II, RCT</td>
<td>40</td>
<td>Improvement in rest pain; reduction in ulcer size</td>
<td>Positive, primary endpoint improved 70%</td>
<td>54</td>
</tr>
<tr>
<td>TALISMAN</td>
<td>PAD</td>
<td>Pl</td>
<td>FGF-1</td>
<td>i.m. injections</td>
<td>Phase II, RCT</td>
<td>125</td>
<td>ulcer healing</td>
<td>Negative (secondary endpoint amputation rate positive)</td>
<td>44</td>
</tr>
<tr>
<td>TAMARIS</td>
<td>PAD</td>
<td>Pl</td>
<td>FGF-1</td>
<td>i.m. injections</td>
<td>Phase III, RCT</td>
<td>525</td>
<td>Time to major amputation or death</td>
<td>Negative</td>
<td>45</td>
</tr>
</tbody>
</table>

CAD = Coronary heart disease  
PAD = Peripheral vascular disease  
RCT = Randomized, controlled trial  
i.a. = intraarterial  
i.c. = intracoronary  
i.m. = intramuscular  
i.my. = intramyocardial  
Ad = Adenovirus  
RV = Retrovirus  
Pl = Plasmid  
ETT = Exercise tolerance test  
TcPO<sub>2</sub> = Transcutaneous oxygen tension  
ST = ECG S-T segment
NA = Not available
# Table 2.

**Ongoing, planned or recent trials in HF, hyperlipidemias, restenosis or vein graft disease**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Disease</th>
<th>Vector</th>
<th>Therapeutic agent</th>
<th>Delivery</th>
<th>Study design</th>
<th>n</th>
<th>Primary endpoint</th>
<th>Main result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CUPID 2</td>
<td>Heart failure</td>
<td>AAV1</td>
<td>SERCA2a</td>
<td>Percutaneous i.c. injection</td>
<td>Phase II, RCT</td>
<td>250</td>
<td>Time to recurrent cardiovascular events</td>
<td>Negative</td>
<td>56</td>
</tr>
<tr>
<td>SERCA-LVAD</td>
<td>Chronic heart failure in patients with LVAD</td>
<td>AAV1</td>
<td>SERCA2a</td>
<td>Percutaneous i.c. injection</td>
<td>Phase II, RCT</td>
<td>24</td>
<td>Safety and feasibility</td>
<td>NA</td>
<td>NCT00534703</td>
</tr>
<tr>
<td>AGENT-HF</td>
<td>Heart failure</td>
<td>AAV1</td>
<td>SERCA2a</td>
<td>Percutaneous i.c. injections</td>
<td>Phase II, RCT</td>
<td>44</td>
<td>Changes in left ventricular end-systolic volume</td>
<td>NA</td>
<td>NCT01966887</td>
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<tr>
<td>STOP-HF</td>
<td>Heart failure</td>
<td>Pl</td>
<td>SDF-1</td>
<td>Percutaneous i.my. helical infusion catheter-mediated injections</td>
<td>Phase II, RCT</td>
<td>93</td>
<td>6 min. walking distance</td>
<td>Negative</td>
<td>58</td>
</tr>
<tr>
<td>RETRO-HF</td>
<td>Heart failure</td>
<td>Pl</td>
<td>SDF-1</td>
<td>Percutaneous retrograde injection via coronary vein</td>
<td>Phase I/II, open-label part and RCT part</td>
<td>52</td>
<td>6 min. walking distance</td>
<td>NA</td>
<td>NCT01961726</td>
</tr>
<tr>
<td>AC6</td>
<td>Heart failure</td>
<td>Ad</td>
<td>Adenylyl cyclase type 6</td>
<td>Percutaneous i.c. injections</td>
<td>Phase I/II, RCT</td>
<td>56</td>
<td>Combined ETT and cardiac function before and during doputamine stress</td>
<td>NA</td>
<td>NCT00787059</td>
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<tr>
<td>Lp(a)</td>
<td>High lipoprotein Lp(a) levels</td>
<td>as-oligonucleotide</td>
<td>as-oligonucleotide against Lp(a)</td>
<td>Weekly s.c. injections</td>
<td>Phase II, RCT</td>
<td>64</td>
<td>Change in plasma Lp(a) concentration</td>
<td>Positive, 67% reduction in plasma Lp(a)</td>
<td>67</td>
</tr>
<tr>
<td>Lp(a)-L</td>
<td>High lipoprotein Lp(a) levels</td>
<td>GalNac-conjugated as-oligonucleotide</td>
<td>liver targeted as-oligonucleotide against Lp(a)</td>
<td>Weekly s.c. injections</td>
<td>Phase I/II, RCT</td>
<td>58</td>
<td>Change in plasma Lp(a) concentration</td>
<td>Positive, 66-92% reduction in plasma Lp(a)</td>
<td>67</td>
</tr>
<tr>
<td>PCSK9</td>
<td>High LDL cholesterol levels</td>
<td>siRNA</td>
<td>siRNA against PCSK9 mRNA</td>
<td>Weekly/monthly s.c. injections</td>
<td>Phase I, RCT (healthy volunteers)</td>
<td>69</td>
<td>Change in plasma LDL cholesterol</td>
<td>Positive, plasma LDL cholesterol reduction 51-60%</td>
<td>68</td>
</tr>
<tr>
<td>------</td>
<td>-----------------------------</td>
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<td>--------------------------</td>
<td>--------------------------------</td>
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<tr>
<td>apoC-III</td>
<td>Hypertriglyceridemia</td>
<td>as-oligonucleotide</td>
<td>as-oligonucleotide against apolipoprotein C-III</td>
<td>Weekly s.c. injections</td>
<td>Phase II, RCT</td>
<td>85</td>
<td>Change in plasma apoC-III level</td>
<td>Positive, apoC-III plasma levels decreased 40-80%</td>
<td>69</td>
</tr>
<tr>
<td>AAV FH</td>
<td>Homozygous familial hypercholesterolemia due to LDL receptor mutation</td>
<td>AAV</td>
<td>LDL receptor</td>
<td>Liver-directed delivery</td>
<td>Phase I, open-label</td>
<td>10</td>
<td>Safety, decrease in plasma LDL cholesterol</td>
<td>NA</td>
<td>NCT02651675</td>
</tr>
</tbody>
</table>

Second part of Table 2
### Third part of Table 2

**Previous trials in HF, hyperlipidemias, restenosis or vein graft disease**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Disease</th>
<th>Vector</th>
<th>Therapeutic agent</th>
<th>Delivery</th>
<th>Study design</th>
<th>n</th>
<th>Primary endpoint</th>
<th>Main result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial hypercholesterolemia</td>
<td>Homozygous familial hypercholesterolemia due to LDL receptor mutation</td>
<td>RV</td>
<td>LDL receptor</td>
<td>Ex vivo gene transfer to patient hepatocytes which were then injected back to the patient</td>
<td>Phase I, open-label</td>
<td>5</td>
<td>Safety, decrease in plasma LDL cholesterol level</td>
<td>Moderate decrease in LDL cholesterol in three patients</td>
<td>2</td>
</tr>
<tr>
<td>Glybera trials</td>
<td>Lipoprotein lipase deficiency</td>
<td>AAV1</td>
<td>Lipoprotein lipase&lt;sup&gt;447X&lt;/sup&gt;</td>
<td>i.m. injections</td>
<td>Phase I/II, open-label</td>
<td>19</td>
<td>post-brandial hypertriglyceridemia severe pancreatitis</td>
<td>Positive, 50% decrease in pancreatitis, improvement in post-brandial chylomicron metabolism</td>
<td>65,66</td>
</tr>
<tr>
<td>Mipomersen trials</td>
<td>Severe hypercholesterolemia</td>
<td>as-oligonucleotide against apolipoprotein B</td>
<td>Weekly s.c. injections</td>
<td>Phase III, RCT</td>
<td>158</td>
<td>Change in plasma LDL cholesterol</td>
<td>Positive, plasma LDL cholesterol reduction 37%</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Italics</td>
<td>In-stent restenosis</td>
<td>as-oligonucleotide against c-myc</td>
<td>Percutaneous i.c. local delivery after stent placement</td>
<td>Phase II, RCT</td>
<td>85</td>
<td>% neointimal volume obstruction by IVUS</td>
<td>Negative</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Prevent III</td>
<td>Vein graft failure in PAD</td>
<td>oligonucleotide</td>
<td>E2F oligonucleotide decoy</td>
<td>Ex vivo pressure-mediated delivery to vein graft</td>
<td>Phase III, RCT</td>
<td>1404</td>
<td>Time to graft reintervention or amputation due to graft failure</td>
<td>Negative</td>
<td>79</td>
</tr>
<tr>
<td>Prevent IV</td>
<td>Vein graft failure in CABG</td>
<td>oligonucleotide</td>
<td>E2F oligonucleotide decoy</td>
<td>Ex vivo pressure-mediated delivery to vein graft</td>
<td>Phase III, RCT</td>
<td>2400</td>
<td>Angiographic vein graft failure</td>
<td>Negative</td>
<td>80</td>
</tr>
</tbody>
</table>
CAD = Coronary heart disease
PAD = Peripheral vascular disease
RCT = Randomized, controlled trial
i.a. = intraarterial
i.c. = intracoronary
i.m. = intramuscular
i.my. = intramyocardial
s.c. = subcutaneous
Ad = Adenovirus
RV = Retrovirus
Pl = Plasmid
as = anti-sense
ETT = Exercise tolerance test
TcPO2 = Transcutaneous oxygen tension
ST = ECG S-T segment
LVAD = left ventricular assist device
IVUS = intravascular ultrasound
NA = Not available
Table 3.

**Trouble shooting of clinical cardiovascular gene therapy trials**

<table>
<thead>
<tr>
<th>General problems</th>
<th>Reasons</th>
<th>Potential solutions</th>
</tr>
</thead>
</table>
| • Lack of clinical efficacy in randomized controlled trials | • Low gene transfer efficiency in target tissues (especially with naked plasmids and intraarterial gene transfer routes)  
• Too low adenoviral or AAV dose in i.m. delivery  
• Short half life of transduced therapeutic factors  
• Difficult or unresponsive patient populations  
• Strong placebo effects | • Intramyocardial or intramuscular gene transfer routes  
• Optimised viral dose, determine dose-response in the clinics  
• Enhance spreading of gene transfer vectors in the target tissues using sufficient injection volumes and number of injections  
• Use of gene transfer as adjuvant therapy in combination with conventional treatments  
• Use of less severely affected patients who can potentially respond to new therapies  
• Randomized, controlled, blinded study design |

<table>
<thead>
<tr>
<th>Specific problems on measured variables</th>
<th>Reasons</th>
<th>Potential solutions</th>
</tr>
</thead>
</table>
| • No detectable effects on clinical symptoms, organ function, metabolism or imaging endpoints | • Too low gene transfer efficiency to cause physiological effects  
• Insufficient distribution of vector or transgene product due to binding to extracellular matrix  
• Regression of new vessels too fast after transient transgene expression  
• Increased blood flow may cause shunting in target tissues  
• Lack of validated, sensitive surrogate endpoints  
• Wrong time points for endpoint measurements  
• Immune responses against vectors or transgenes  
• Pathophysiology still incompletely understood | • Perfusion measurements shortly after gene transfer to show proof-of-concept  
• Careful dose optimization in preclinical and early clinical studies  
• Use of gene transfer vectors that produce long term and/or regulated gene expression  
• Development and validation of more sensitive surrogate endpoints for imaging, organ function and target tissue metabolism  
• Understanding and controlling immunoresponses against vectors and transgenes  
• Combination of gene therapy with established treatment procedures |