There are several reasons why new therapeutic strategies need to be developed for coronary artery and peripheral artery disease. Despite major advances in medical treatment, interventional cardiology and surgery, particularly over the past 10 years, arterial disease continues to be one of the most frequent causes of death and morbidity. In addition, lesion or patient-related factors currently restrict the use of interventional and surgical techniques in some patients.

Thus, the aims of this article are 1) to identify how current revascularization techniques might be improved by new therapy strategies, 2) to summarize some of the most recent experience in gene therapy, 3) to illustrate that gene therapy might have potential in managing restenosis and inducing angiogenesis, and 4) to discuss any advantages of gene therapy over conservative/standard therapy.

Identification of novel strategies for coronary artery disease and critical peripheral artery disease. The most common form of treatment for coronary artery disease and critical peripheral artery disease is presently revascularization, carried out either percutaneously via angioplasty or surgically.

Goals of new therapeutic strategies. New therapeutic strategies for vascular disease may target any aspect of the pathophysiology of coronary artery or peripheral disease. If the success of percutaneous techniques such as angioplasty or stenting could be increased, then this would benefit a significant amount of patients. Alternatively, myocardial or peripheral ischemia could be reduced if local blood supply could be increased by other methods. Hence, two important therapeutic strategies might be: 1) to modify the pathological process of restenosis in new ways by targeting any of the factors detailed above, and/or 2) to increase the formation of collaterals through therapeutic angiogenesis, consequently improving the perfusion of the heart or the ischemic limb. Such strategies for coronary and peripheral artery disease may be used as sole therapy or in combination with standard procedures.
One of the ways in which targeting restenosis might be achieved is through gene therapy. Any stage of the restenosis process might be targeted, but most investigations have so far been directed at cell proliferation. Alternatively, or in combination with gene therapy, mechanical methods of preventing restenosis are under development; for example, the use of sophisticated local drug delivery strategies and biodegradable stents. The use of new materials in combination with gene therapy may increase the value of stent technology.

Gene transfer has now been carried out in more than 4,000 patients as part of over 400 registered protocols in the U.S. and Europe since 1989. Although success rates vary and many of the efficacy and safety issues remain unsolved, the potential of this alternative therapeutic method is well accepted. Dozens of clinical trials into conventional pharmaceutical agents have not succeeded in reducing restenosis rates postangioplasty or in inducing angiogenesis in chronic occlusive disease. This has encouraged the development of gene therapy for this purpose. The introduction of special catheters for local drug delivery allows high efficacy combined with maximum safety for the introduction of gene therapy. This allays many of the fears associated with the technique.

Most of the early gene therapy investigations on the vasculature manipulated vascular cells ex vivo with the aim of subsequently administering these to animals with arterial injury. Alternatively, products that alter genes can be administered directly, where the aim is to locally modify certain cells in vivo, and this has become the preferred approach in the cardiovascular field, using local drug delivery devices as discussed below.

The use of gene transfer for the prevention of restenosis. The site of a lesion in the arterial wall is accessible percutaneously by local drug delivery devices, which can achieve high regional concentrations of transferred agents, including genes. If suitable drug application is performed at the time of angioplasty, no further intervention may be necessary. This knowledge has been used to develop several strategies targeting the pathological process of restenosis. Within the vasculature, the aim is generally either to reduce cell proliferation or matrix formation, to induce angiogenesis, or maybe to influence remodeling.

Early research into the pathology of the restenosis process focused mainly on the histological changes in the arterial wall after injury and on the distribution of extracellular proteins, particularly growth factors. This did not provide much information about their function. Fresh restenosis tissue has been used to perform contemporary molecular techniques at the transcriptional level, identifying important genes that control differentiation, cell replication and angiogenesis that might be modified to affect restenosis. For restenosis, the cells targeted are commonly proliferating endothelial or vascular smooth muscle cells. Cell proliferation may be altered at the extracellular level, which includes growth factors, or in cells themselves, where signal transduction leads to activation of the cell cycle and cell division.

In principle, therapy in vivo targeting cell proliferation can be aimed at inhibiting normally occurring growth stimulators or augmenting natural growth inhibitors. Alternatively, therapy can intend to kill cells with the introduction of cytotoxic therapy. In general, the lower the signal transduction cascade cell regulation is influenced, the more predictable are the effects achieved, which therefore minimizes adverse events.

Clinical trials using gene transfer for the prevention of restenosis. Local delivery of the gene for VEGF using a VEGF plasmid was first used clinically by Jeffrey Isner in the U.S. It was delivered via a hydrogel-coated balloon catheter following percutaneous transluminal angioplasty of peripheral arteries. To date, more than 13 patients have been included in this placebo-controlled double-blind trial and so far no restenosis event has been documented. In Europe, at least 55 patients undergoing peripheral angioplasty have been treated with gene therapy in Finland by Seppo Ylil-Herttuala. Local vascular gene transfer was carried out via a infusion perfusion catheter (the Dispatch catheter) following peripheral angioplasty. Eight patients underwent with adenoviral transfer of LacZ (a non-therapeutic reporter gene) and 47 patients underwent liposomal-mediated or adenoviral transfer of the gene for VEGF in a controlled, randomized and double-blind trial. Results from this trial are not yet available; however, in another controlled clinical trial by the same group, the gene for VEGF was delivered by the Dispatch catheter and transferred using liposomes into 15 patients. The application of plasmid of VEGF did not reduce restenosis rates.

A gene therapy decoy strategy has been tested in a randomized trial in patients undergoing vein grafting for peripheral vascular disease. Oligonucleotides that prevent the action of the transcription factor E2F, a factor that normally activates genes for proteins that stimulate the cell cycle, were delivered locally into the peripheral vein graft during the surgical procedure. There was reduced local cell proliferation and intimal hyperplasia, with fewer graft occlusions, revisions, or critical stenoses in the treated E2F-decoy group than in the untreated group at one year follow-up. The use of the E2F decoy to prolong the patency rates of coronary bypass grafts has been investigated in eight patients. There was increased patency in the vein grafts that underwent pressure transsection prior to implantation, and tissues demonstrated reduced proliferation rates.

For more successful in vivo gene therapy, particularly for restenosis, higher gene transfer efficiencies may be needed. Although viral gene transfer has been considered the best for transfer efficiency, there are safety issues for staff with this type of transfer, and non-viral gene transfer may be preferable. So far, retroviral gene therapy has mainly been used for ex vivo gene therapy, but adenoviral gene transfer has particular biosafety problems, with relatively low efficacy in patients because of the presence of...
adeno viral antibody in significant titers and side effects related to the pathogenicity of adenoviruses for humans, resulting in local inflammatory reactions. Recent developments with novel liposomes and adjuvant agents allow non-viral gene transfer rates in vivo to approach gene transfer rates achieved through adenoviral gene transfer.17

Alternatively, further improvement may be achieved searching for new therapeutic gene targets. Ideal would be gene products secreted by transfected cells for a bystander effect, aiming at halting cell replication without killing the cell. A bystander effect occurs when a gene or resultant protein is transferred from transfected cells and affects non-transfected neighboring cells. Thus, a low transfection efficiency may still result in a significant effect. One such new agent that demonstrates successful inhibition of intimal hyperplasia formation is the insect antibiotic cecropin.18,19

The use of gene transfer to induce angiogenesis. Angiogenesis, which is the increased formation of collateral vessels under the influence of angiogenic growth factors, was first described about 30 years ago. Since then, many positive endogenous regulators of angiogenesis have been described in the literature. They include peptide growth factors, multifunctional cytokines and immune mediators, chemokines, enzymes, hormones, oligosaccharides and hematopoietic growth factors.20

In this field, vasculogenesis represents the de novo differentiation of mesenchymal progenitor cells into endothelial cells, angiogenesis is the sprouting of new capillaries from pre-existing vessels and arteriogenesis is the formation of new collaterals based on pre-existing arteriolar anastomoses. Vasculogenesis, angiogenesis and arteriogenesis probably differ in their molecular mechanisms and are very likely to be mediated by differential effects of several growth factors.21 During embryonal development, the formation of vessels includes vasculogenesis and angiogenesis. In the post-natal organism, angiogenesis predominates, for example, in the female reproduction cycle or during wound healing. Pathological processes such as tumor growth and the formation of metastases also require neovascularization.

The identification of angiogenic growth factors has allowed the development of new strategies for the treatment of chronic vessel occlusions. Therapeutic new vessel generation is a potential alternative therapy for patients with therapy-refractory angina or critical ischemia of the lower limb. Relief of symptoms results from both angiogenesis and arteriogenesis.

Clinical trials: Gene therapy for angiogenesis induction

Peripheral artery disease. So far, only the genes for peptide growth factors have been clinically studied. Investigations in study animals (described above) demonstrated several potentially beneficial effects which might be useful in human peripheral artery disease. The first published clinical trials in patients used predominantly the gene for VEGF165, applied using naked plasmid DNA, liposome-complexed DNA or adenoviral vectors. Several clinical gene therapy trials for the treatment of vascular disease using the gene for VEGF have been approved by the Recombinant DNA Advisory Committee of the National Institutes of Health in the U.S.; more trials have been conducted in Europe. The majority of such trials aim to improve ischemia in severe peripheral artery disease.

Local delivery of the gene for VEGF has been performed via a hydrogel-coated balloon catheter or by subcutaneous delivery.22 Almost 200 patients treated with gene therapy for cardiovascular disease have been published so far and promotion of angiogenesis with improvement of blood perfusion has been demonstrated, predominantly at relatively high dosages.23 Side effects observed were edema of the leg treated and a transient increase in blood levels of VEGF, which may potentially promote tumor growth (although this has not been reported to have occurred). More localized therapy may be advantageous. A secondary aim in these cardiovascular gene therapy trials has been to prevent restenosis following percutaneous transluminal angioplasty of peripheral arteries, again via a catheter-based approach.24 As the plasmid DNA is applied locally, this minimizes side effects, and reports concur with this so far.

Most human investigations that have been carried out have been phase I or phase I/IIa trials, which predominantly test the safety of the treatment, and as a second aim, but less importantly, may also look at the efficiency of the treatment with the genes for VEGF isoforms. At least 55 patients with critical limb ischemia (Rutherford score 4–6) have been given pHVEGF165 intramuscularly. The dose used was 0.1–2.0 mg and 2–4 mg plasmid. All trials demonstrated that the use of intramuscular gene transfer with naked plasmid DNA was safe. In some patients, gene therapy resulted in clinical improvement, demonstrated by an increase in perfusion in the treated limbs, measured as ankle-brachial index, and by improved healing of ischemic ulcers. A decrease in the size and number of ulcers was evident in some of the cases. In some patients, amputations previously seen as necessary were avoided and in other patients planned amputations could be more limited.

In a trial of 22 patients, improvement of ischemia-associate neuropathy was observed 3 months later. Objective parameters, such as nerve conduction, were significantly improved 6 months following transfer of the gene for VEGF; for diabetic neuropathy, the results were similarly good. Plasmid DNA was mostly injected locally into skeletal muscle of the ischemic limb. Increased levels of VEGF protein was detectable in plasma using ELISA techniques 1–3 weeks following intramuscular gene application. Despite this increase in VEGF protein levels, there was no progression of diabetic retinopathy, nor was there any development of new tumors not diagnosed at the time of patient recruitment and screening (both of these complications were predicted by researchers). Increased collateralization induced by gene therapy was detected solely in the ischemic limb, but
not in any of the non-ischemic tissues. The most likely explanations for the localized effect are the increased receptor expression for VEGF isoforms in ischemic tissues and the short half life of the VEGF protein in blood plasma. Transient peripheral edema through increased vascular permeability induced by VEGF was the most frequent unwanted side effect; however, this usually did not require specific therapy.

A multicenter trial on the effects of the gene for VEGF-1 on critical ischemia is being conducted in patients with critical limb ischemia in Finland by Seppo Ylä-Herttuala. Liposome-complexed plasmid for VEGF-1 is released from the adventitia via a biodegradable reservoir. In patients with inflammatory thrombangiitis obliterans (Buerger’s disease), complete healing of ischemic ulcers was observed in 3 limbs, and an improvement of the ankle-brachial index with improved angiographic collateralization in all treated limbs was observed following two intramuscular applications of the VEGF165-plasmid (2 and 4 mg DNA) into 7 treated limbs in 6 patients.

Results were similar when the plasmid pVGI.1 encoding the VEGF isoform VEGF-2 was used. Rest pain, frequency of pain episodes and the use of analgesics were reduced in some patients; clinical worsening, frequently observed in progressive stages of the disease, leading to amputations, was prevented in many cases. Peripheral edema was less frequent compared with the use of VEGF-1. Preliminary analysis of 13 patients demonstrated improved collateralization and perfusion of the treated limbs following application of the gene for VEGF-2 in a placebo-controlled trial (3:1).

**Coronary heart disease.** Similar to the clinical investigations carried out in patients with peripheral artery disease, recent studies with the plasmids pVEGF165 and pVGI.1 (VEGF2) have established proof of concept that intramyocardial administration of naked DNA in the form of a plasmid encoding VEGF protein results in angiographic evidence of augmented collateral artery development in patients with chronic myocardial ischemia. In studies with the VEGFI plasmid pHVEGF165, as well as as the VEGF-2 plasmid pVGI.1, patients experienced decreased angina and showed evidence of improved myocardial perfusion by single photon emission computed tomography (SPECT) after gene transfer, without concomitant adverse effects.24-26

Direct intramyocardial application of the gene via a left anterior thoracotomy was the approach for the first gene applications for the treatment of therapy-refractory angina in advanced coronary heart disease, requiring a surgical procedure with anesthesia in such high-risk patients. Subsequently, a less invasive transluminal interventional procedure has been developed, allowing in vivo real time mapping of the left ventricle, as well as gene application via a similar mapping catheter. Selected patients have already been treated, demonstrating that this approach is safe and effective.77

**Alternative therapeutic genes.** Other multicenter angiogenesis trials are currently investigating the effect of vectors encoding the gene for FGF-4 (using adenovirus, intramuscular transfer) and FGF-1 (as plasmid, using intramuscular transfer) in Europe (Collateral Therapeutics/Schering AG) as well as in the U.S. (RPR Gencell). Acidic and basic FGF (aFGF, bFGF) have also been used for angiogenesis trials in humans; however, these have mostly been applied as the biologically active protein and not the gene.

**Complications in clinical gene therapy trials for arterial disease.** Five deaths have so far been reported during the 1-year follow-up period of vascular gene therapy trials (personal communication, CATO Research/Vascular Genetics Inc.). These were classified by the investigators as non-therapy-associated. Patients with critical limb ischemia often suffer from generalized atherosclerosis, including coronary heart disease as well. The 1-year mortality in such patients with critical limb ischemia is 26% and the 5-year mortality is less than 50%. Patients with therapy-refractory angina often also have ischemic cardiomyopathy, further limiting life expectancy, with a predicted 1-year mortality of around 20%. Therefore, many patients eligible for ongoing vascular gene therapy trials are at high risk of dying naturally during follow-up. Preliminary analyses of VEGF or placebo-treated patients show equal distribution of deaths in the gene-treated and placebo groups (personal communication, CATO Research USA/Vascular Genetics Inc.).

**Pros of cardiovascular gene therapy, including safety aspects.** Therapeutic angiogenesis has particular advantages in the treatment of patients with advanced chronic arterial occlusive disease who otherwise have no option for surgical or interventional arterial revascularizations with satisfactory mid- or long-term results. Directed growth of new vessels in a similar fashion to the naturally occurring beneficial collateralization that sometimes is seen could be particularly important for improving the management of patients with arterial occlusive disease in the context of further diseases, such as diabetes, microangiopathy or infrapopliteal ischemia. The induction of new collaterals and capillaries in the right place may lead to a permanent clinical improvement, with a reduction in the rate of amputations and with a beneficial effect on the limited prognosis.

With regard to targeting particular proteins, recombinant proteins or gene therapy strategies may both potentially be of use. The advantages of gene therapy include better tolerance, as a significantly smaller amount of substance is applied, reduced antigenicity and potentially a longer lasting yet transient expression of the relevant vascular growth factors or growth factor inhibitors. Viral vectors increase transfection efficiencies and therefore appear to offer a theoretical advantage. However, high transfer efficiencies are less important when gene products such as VEGF are targeted, as they include signal sequences which allow active secretion from intact cells and therefore lead to a paracrine or bystander effect. Gene therapy strategies may also minimize systemic side effects such as blood pressure depression (VEGF) or nephrotoxicity (bFGF) seen in some...
Research is gradually increasing our knowledge about the pathology of restenosis, and we have learned a great deal about potential targets in recent years. New targets are still being identified for treating arterial damage from stored tissue specimens and in vitro studies. Investigations into the importance of thrombus, extracellular matrix, remodeling and apoptosis may provide evidence of other regulating molecules that may be targeted. The further recognition of positive-acting factors leading to cell growth, and negative-acting factors leading to growth suppression and apoptosis, will increase the targets available for testing. Suitable targets may include cell cycle inhibitors, homeobox factors, angiogenic VEGF or certain cytotoxic foreign genes which can also slow down physiological cellular functions instead of killing cells.

It is anticipated that there will be significant synergy between improving gene therapy technology and the ongoing Human Genome Project, which will provide additional targets that could be used for gene therapy to the vascular wall.

Improvement in gene therapy strategies are likely to be important in targeting the vasculature. Naked DNA may be used if gene products are secreted, as low transfection efficiencies seem to be sufficient. However, if gene products remain intracellular, potent vectors, including recombinant viruses, may be needed. The recent use of lentiviral vectors (a subfamily of retroviruses) for gene therapy has expanded the possible cell types that can be infected. It is also possible to ensure that a therapeutic gene product is only made in the targeted cells by the use of tissue-specific promoters, DNA sequences that determine the site of transcription initiation. Knowledge of target cell properties can allow tailoring of the promoter region so that the expression of therapeutic genes is restricted to the target cells, increasing safety. For example, specific promoters could restrict gene expression to cells in a particular stage of differentiation or allow gene transcription to be turned on within a limited time frame; as a result, there is more control over the synthesis of the therapeutic protein. Promoters could also be locally radiation-induced or drug-responsive.

Conclusions. Various aspects of gene therapy are currently under investigation to further solve vascular problems. These include novel targets within the pathophysiology of the process, novel methods of targeting by gene therapy and the development of local approaches, including special catheters and stents.

Gene transfer for the suppression of restenosis or the induction of therapeutic angiogenesis represents an alternative treatment for patients with advanced atherosclerosis. Numerous animal experiments have demonstrated the safety and efficacy of various gene therapy strategies (predominantly targeting VEGF isoforms). The advantages of gene therapy strategies include minimization of systemic side effects and the slow release of encoded factors, detectable for several months, allowing long-term antiproliferative or
angiogenic effects. The demonstration of vascular gene transfer using different vectors has been demonstrated in predominantly uncontrolled clinical phase I trials at single centers. There was a tendency to clinical improvement, indicating the efficiency of the gene therapy. Results of placebo-controlled and double-blind multi-center trials are not yet available.

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REFERENCES

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