

# GENE TRANSFER FOR ANGIOGENESIS IN CORONARY ARTERY DISEASE

---

Roger J. Laham,<sup>1,2</sup> Michael Simons,<sup>1</sup> and Frank Sellke<sup>1,3</sup>

<sup>1</sup>Angiogenesis Research Center, <sup>2</sup>Interventional Cardiology Section, Department of Medicine, and <sup>3</sup>Cardiothoracic Surgery Division, Department of Surgery, Harvard Medical School and Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, Massachusetts 02215; e-mail: rlaham@bidmc.harvard.edu, fsellke@bidmc.harvard.edu

**Key Words** ischemia, atherosclerosis, revascularization, no-option patients, local delivery

■ **Abstract** Angiogenesis is a promising novel therapeutic strategy to provide new venues for blood flow in patients with severe ischemic heart and peripheral vascular disease, who are not candidates for standard revascularization strategies. We describe the underlying mechanisms involved in physiologic and therapeutic angiogenesis, underscoring the relative importance of vasculogenesis, angiogenesis, and arteriogenesis. We then present the various gene transfer vectors including plasmid, viral, and cell-based vectors, and various delivery modalities. The available preclinical data are presented, followed by a description of preliminary clinical experience, with an emphasis on the preliminary nature of these results, which address safety and not efficacy. Finally, we discuss the promises and pitfalls of clinical angiogenesis and gene transfer studies, stressing the importance of proper design of clinical trials and adequate protection of research subjects.

## INTRODUCTION

Ischemic heart disease remains the leading cause of death in the western hemisphere. Coronary artery disease, which causes ischemic heart disease, caused 466,101 deaths in the United States in 1997, 1 of every 5 deaths (1).

In 2000, an estimated 1,100,000 Americans will have a new or recurrent coronary attack (defined as myocardial infarction or fatal coronary artery disease). Twelve million Americans alive today have a history of heart attack, angina pectoris, or both. From 1987 to 1997, the death rate from coronary artery disease declined 24.9% (1a, 2). Although the most effective treatment strategy would be to prevent coronary artery disease, aggressive risk-factor modification (including the control of hypertension, diabetes mellitus, tobacco use, and elevated cholesterol) has produced only a modest decline in the incidence of this devastating illness (3-8). Therefore, efforts have focused on the treatment of coronary artery disease.

Traditional treatment strategies include risk-factor modification, use of antiplatelet agents, reduction of myocardial oxygen demand, coronary vasodilation, and restoration of myocardial perfusion using percutaneous coronary intervention (PCI) and coronary artery bypass surgery (CABG). However, up to 20%–37% of patients with ischemic heart disease either cannot undergo CABG or PCI, or receive incomplete revascularization with these standard revascularization strategies (9–15). Many of these patients have residual symptoms or myocardial ischemia despite maximal medical therapy. Therefore, such patients require an alternative revascularization strategy to relieve angina and to combat myocardial ischemia. Therapeutic angiogenesis may fill this role by providing new avenues for blood flow (11, 12, 15). Therapeutic angiogenesis is the focus of this review as a paradigm for coronary artery disease. Other therapeutic targets include reduction of familial hyperlipidemia, lipoprotein-a deficiency, hypertrophic cardiomyopathy, myocardial diseases (dystrophy, cardiomyopathy), and congenital malformations.

## GENE THERAPY VECTORS

When naked DNA comes into contact with the cell membrane, only a small amount enters the cell, leading to relatively low gene-transfer efficiency (16, 17). Therefore, a carrier or a virus vector is generally used to increase transfection efficiency and achieve adequate expression of the therapeutic molecule. Table 1 lists the gene therapy vectors discussed in this review.

### Nonviral Vectors

Naked plasmids or liposomal DNA (18–23) complexes are the most commonly used carrier molecules. However, only a small fraction of plasmid DNA enters the nucleus, where it persists in an episomal location (not integrated into the genome), resulting in limited-duration transgene expression in both proliferating and nonproliferating cells. Although transgene expression has been reported to persist as long as 3–4 weeks, in most cases the duration is far shorter. The production and scale-up of plasmid and liposomal complexes are relatively easy, but the low efficiency of transfection, as well as the short duration and low levels of transgene expression, limit this approach. Nonviral vectors may not be sufficient to achieve therapeutic concentrations of the protein product. However, these vectors may be very effective for induction of the desired immune response (“DNA vaccine”).

Phospholipid formulas such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-dodecanoyl/1,2-dioleoyl-sn-glycero-3-phosphocholine and cationic polymers such as polyL-ornithine with galactose and the fusigenic peptide mHA2

**TABLE 1** Gene transfer vectors

<b>Vector</b>	<b>Advantages</b>	<b>Limitations</b>
Plasmid	Good safety profile Episomal location Easy to produce	Low transfection efficiency Short transgene expression
Adenovirus	High transfection efficiency Episomal location Easy to produce Large expression cassette	Transient duration of expression Inflammatory reaction
AAV*	Good transfection efficiency Episomal location Longer transgene expression	Difficult to produce and scale up Small expression cassette
Retrovirus	Chromosomal integration Long transgene expression Low titers	Transfection limited to dividing cells Long transgene expression Production of replication—competence Inactivation of LTRs*
Cell-based delivery	Prolonged expression Regulatable expression Multiple transgene expression	Prolonged expression Difficult to produce and scale up Local inflammatory reaction

\*AAV, adeno-associated virus; LTR, retroviral long terminal repeats.

(Galactose-polyL-ornithine-mHA2) can improve the transfection efficiency (23, 24). In addition, Epstein-Barr virus (EBV)-based expression plasmids may prolong gene expression at therapeutic levels, and can efficiently and repeatedly re-transfect immunocompetent hosts.

## Viral Vectors

Adenoviruses enter cells via specific receptors with subsequent lysosomal degradation. They release viral DNA into the cytoplasm, which makes its way to the nucleus, remaining in the extrachromosomal location. Replication-deficient adenoviruses are produced *in vitro* in specific packaging cells that complement gene products (E1, E3) deleted from the viral genome to prevent *in vivo* replication (25–31). Adenoviruses have the advantages of easy production in high titers, relatively high transduction efficiency, and the ability to infect and replicate both proliferating and nonproliferating cells (Figure 1). However, both first- and second-generation adenoviral vectors are associated with a significant local inflammatory reaction that eventually extinguishes transgene expression. Circulating antiadenoviral antibodies, common for some adenoviral subtypes, greatly reduce duration and magnitude of expression. Newer encapsidated (guttated) adenoviruses may produce milder inflammatory reactions (32–34). These modifications also

increase the capacity of these adenoviruses, allowing them to carry long pieces of DNA such as the dystrophin gene (33).

Recombinant adeno-associated viruses (AAVs) are promising candidates as gene vectors because they transduce nondividing cells and permit lasting transgene expression in a wide spectrum of tissues (35–44). However, AAVs are difficult to produce, and they have a small expression cassette (39, 41). Newer procedures for high-throughput production, screening, and characterization of AAV vectors may circumvent the scalability problem (36), and the size limitation may be overcome by using a dual-vector approach (39–41, 44).

Retroviruses enter cells via specific receptors, after which viral RNA is reverse transcribed to DNA that is then integrated into the cellular chromosomal architecture. This integration results in stable, prolonged, and potentially high (depending on integration site) expression of the therapeutic transgene (45–49). Replication-deficient retroviruses are produced *in vitro* in specific packaging cell lines containing retroviral genes (G, P, E) that have been deleted from the retroviral genome. Retroviruses can only transduce dividing cells, which limits their target cell population. They are further limited by their low titer. In addition, the efficient insertion of genes by retroviruses is often complicated by transcriptional inactivation of the retroviral long terminal repeats (LTRs) and by the production of replication-competent retroviruses.

These and other difficulties are being resolved by using modular vectors, in which the desirable features of different vector systems are combined. Examples of synergistic vectors include virosomes (liposome/virus delivery), adeno-retro vectors, and MLV/VL30 chimeras (49). The development of lentivirus vectors has allowed efficient gene transfer to quiescent cells (33, 45, 47, 50, 51), and the development of pseudotyping has increased viral titers (52). Other gene-transfer vectors are being developed to circumvent all the pitfalls of currently available vectors (26, 31, 53–58).

## Cell-Based Gene Transfer

Cell-based gene transfer is a promising new strategy that utilizes autologous cells transfected with a transgene of interest to express that transgene *in vivo*. The use of autologous cells circumvents the inflammatory response. Such a system achieves prolonged expression by stable transfection of the desired gene through various measures, including *in vitro* retroviral or lentiviral transduction (59–67). In addition, complex multigene constructs can be generated that allow stable, regulatable transgene expression.

To illustrate this delivery modality, endothelial cells were evaluated for their potential use in gene transfer to deliver apolipoprotein E (apoE) in apoE knockout mice (59). After transplantation of the apoE-secreting Pro-175 endothelial cells into apoE-deficient mice, serum cholesterol levels were lower in animals that had received the apoE-secreting endothelial cells than in age-matched controls that had

received nonsecreting endothelial cells. Concomitant with cholesterol reduction, atherosclerotic aortic plaques were noticeably reduced in the animals that received apoE-secreting endothelial cells.

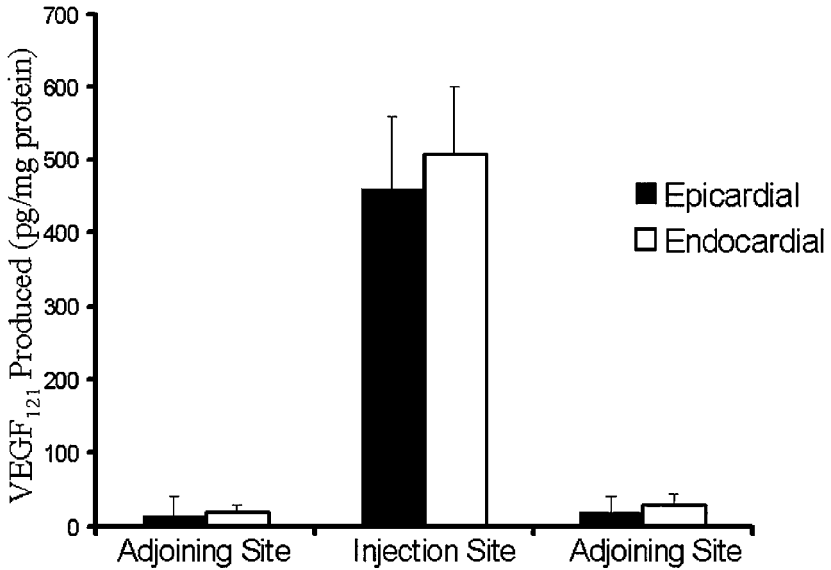
In other studies, cell lines were transduced with a retroviral vector containing the human erythropoietin (hEpo) cDNA driven by the hypoxia-responsive promoter (61). *In vitro*, these cells showed a threefold increase in hEpo secretion as oxygen levels were shifted from 21% to 1.3%. Serum hEpo levels in animals exposed to 7% oxygen were twice as high as in their control counterparts, which were kept at 21% oxygen.

### Specific Requirements for the Cardiovascular System

Sustained expression is desirable for cardiovascular diseases with a specific gene defect, such as familial hypercholesterolemia, various dystrophies, or Lpa deficiency. On the other hand, transient expression is preferable for cardiovascular conditions that are self-limited, such as restenosis after coronary angioplasty. Therapeutic angiogenesis would be best accomplished with a transient expression vector, since sustained expression of angiogenic cytokines may lead to pathological angiogenesis (62–68).

### DELIVERY OF GENE-TRANSFER VECTORS

Although a tremendous amount of investigation has been performed to design and perfect gene-transfer vectors and even more work has gone into identifying potential target molecules for gene transfer, the question of optimal delivery has scarcely been explored. The vasculature and the myocardium are among the easiest targets for gene transfer because of their accessibility and the need for only transient expression. Although it was thought that getting the vector in contact with its target would be sufficient, a whole series of steps were being taken for granted. The vasculature and the myocardium are subject to rapid blood flow, which leads to washout of the vectors after only a brief period of contact with target cells. Intracoronary delivery results in significant systemic recirculation, exposing nontarget organs to the vector (69). Intramyocardial delivery, using the endocardial catheter-based approach or the epicardial open-chest approach, appears to have similar transfection efficiency (Figure 2), thus obviating the need for open thoracotomy. However, the equipment used to deliver these vectors may inactivate the viral and plasmid particles, a possibility that has not been adequately studied. For example, catheter-based delivery appears to result in significant inactivation of adenoviral particles, which is proportionate to the residence time in the catheter (time between filling catheter and injection). Catheter-based plasmid delivery significantly reduces the transfection efficiency of plasmid vectors, which is proportional to the injection speed and pressure. Of note, all these experiments were performed well after clinical trials with these vectors.



**Figure 2** Intramyocardial delivery of an adenoviral construct encoding VEGF<sub>121</sub> via epicardial delivery (open thoracotomy and injection using a 25-gauge needle of 0.1 ml), and endocardial delivery of the same volume containing  $10^{10}$  PFU viral particles using the Biosense<sup>R</sup> myostar<sup>R</sup> injection catheter with Biosense<sup>R</sup> NOGA guidance. VEGF<sub>121</sub> levels were measured using ELISA for the injection sites and for adjoining sites and showed similar VEGF<sub>121</sub> expression with both intramyocardial injection modalities.

## THERAPEUTIC ANGIOGENESIS

Angiogenesis is a complex process that involves stimulation of endothelial cell proliferation and migration, stimulation of extracellular matrix breakdown, attraction of pericytes and macrophages, stimulation of smooth muscle cell proliferation and migration, formation and “sealing” of new vascular structures, and deposition of new matrix (12, 68, 70, 71). It is likely that this process requires a coordinated action of several mitogens and cascades. Gradual occlusion of coronary arteries is frequently associated with development of collateral circulation in patients with atherosclerosis (72–78). Although the existence of collateral circulation in such patients is associated with improved clinical outcomes, the net effect is rarely adequate to compensate for the flow loss that results from occlusion of native epicardial coronary arteries (73, 75, 77–79). The large number of revascularization procedures performed (375,000 CABG procedures and >500,000 PTCA procedures annually in the United States alone) attests to the inadequacy of native collateralization.

Myocardial ischemia is a potent angiogenic stimulus. A number of growth factors have been isolated from the ischemic myocardium, suggesting that these

molecules may play a role in ischemia-induced angiogenesis. Among these growth factors, fibroblast growth factors [acidic (aFGF, FGF1) and basic (bFGF, FGF2)] and vascular endothelial growth factor (VEGF) are the most widely studied and have successfully induced angiogenesis in animal models (68, 80–86).

Three different processes may contribute to the growth of new blood vessels: vasculogenesis, arteriogenesis, and angiogenesis (87, 88).

Vasculogenesis refers to formation of new vessels from pluripotent stem cells, which, in the course of embryonic development, form primitive vascular plexus (89). Although preliminary evidence suggests that vasculogenesis may play a role in new-vessel formation in mature tissues (90), the significance and frequency of this event has not been well defined.

Angiogenesis, which occurs in mature tissues, is responsible for formation of new vessels lacking developed tunica media from post-capillary venules. Capillary proliferation in healing wounds or along the border of myocardial infarction are examples of angiogenesis (91).

Arteriogenesis is responsible for the formation of arteries possessing fully developed tunica media. Arteriogenesis includes a poorly understood series of events that may involve maturation of preexisting collaterals or the *de novo* formation of vessels with proliferation and maturation of various cell types including endothelial cells, smooth muscle cells, and pericytes (87). Angiographically visible collaterals in patients with advanced obstructive coronary or peripheral vascular disease are examples of arteriogenesis.

Although related, arteriogenesis and angiogenesis may be induced by different stimuli. Thus, arteriogenesis occurs generally proximally, far removed from the ischemic territory and thus is probably not directly induced by hypoxic/ischemic stimuli. Other factors, such as hemodynamic shear stress and local prothrombotic changes, have been suggested (87). On the other hand, angiogenesis, occurring on the edges of ischemic/damaged tissues, is almost certainly controlled by tissue hypoxia.

Both angiogenesis and arteriogenesis require a local inflammatory milieu. This involves the presence of blood-derived macrophages that secrete inflammatory mediators, such as IL-1 $\alpha$ , TNF- $\alpha$ , CXC chemokines, hypoxia-induced factor (HIF)-1 $\alpha$  regulating peptides (e.g. PR39), and various matrix proteases (92–96). Systemic suppression of macrophage function may adversely affect ability to induce new vessel growth (95, 97).

A number of growth factors have been evaluated for their angiogenic potential. These include fibroblast growth factors, VEGFs, hepatocyte growth/scatter factor (HGF/SF), chemokines (e.g. IL-8 and MCP-1), growth factors involved in maturation of the vascular tree [e.g. angiopoietins and platelet-derived growth factor (PDGF)] (68, 79), and transcription factors that stimulate expression of angiogenic cytokines and their receptors (e.g. HIF-1 $\alpha$ ) (98, 99).

Two approaches have been used to achieve therapeutic angiogenesis: gene transfer and protein therapy. Protein therapy's advantages include controlled delivery, established safety, predictable pharmacokinetics and tissue therapeutic levels, and

absence of long-term unexpected side effects (68, 69, 100). The perceived disadvantages of this strategy include the short tissue half-life of most proteins and the high cost of recombinant molecules, shortcomings that could be addressed by sustained delivery if that is needed. Experience with protein-based (growth factor) therapy has been more extensive than with gene transfer; several phase I and phase II double-blind placebo-controlled studies of protein therapy have been performed to date (15, 100–102). Some angiogenic agents cannot be delivered as proteins and thus may necessitate gene transfer. Example of such agents are HIF-1 $\alpha$  and PR39 (96, 103–105). However, for FGFs and VEGFs, protein therapy may supersede gene transfer, especially given the limitations of current vectors.

Several preclinical studies investigated gene transfer of angiogenic growth factors. Replication-deficient adenovirus vector AdCMV.VEGF<sub>165</sub>, with the cytomegalovirus (CMV) promoter driving a VEGF-A<sub>165</sub> cDNA, was used to transduce human umbilical-vein endothelial cells (HUVEC) and rat aorta smooth muscle infected cells (RASMC) (106). Infection with AdCMV.VEGF<sub>165</sub> resulted in enhanced proliferation of HUVEC and tube formation on Matrigel *in vitro*. In an *in vivo* mouse Matrigel assay, AdCMV.VEGF<sub>165</sub> enhanced gel-plug (Matrigel) neovascularization relative to AdCMV. $\beta$ -Gal control vector (106). The same group studied the angiogenic potential of AdCMV.FGF-1 (secreted and nonsecreted forms) and found increased angiogenesis *in vitro* (HUVEC) and *in vivo* (mouse Matrigel assay) (86). In an ameroid constrictor model of stress-induced myocardial ischemia, intracoronary injection of a recombinant adenovirus expressing human fibroblast growth factor-5 (FGF-5) resulted in the appearance of messenger RNA and protein expression of the transferred gene (85). Two weeks after gene transfer, regional abnormalities in stress-induced function and blood flow were improved, effects that persisted for 12 weeks. Improved blood flow and function were associated with evidence of angiogenesis (85).

The feasibility of a single administration of a replication-deficient adenovirus vector encoding the cDNA for human VEGF<sub>121</sub> (AdCMV.VEGF<sub>121</sub>) was evaluated in retroperitoneal adipose tissue (107). *In vivo* quantification of the number of blood vessels, using 30 $\times$  visualization of the adipose tissue, demonstrated an increase in vessel number within 10 days, which plateaued by day 30 with a 123% increase in vessel number compared with the control vector. One study found that plasmid phVEGF<sub>165</sub> vector, applied to the polymer coating of an angioplasty balloon and delivered percutaneously to the iliac artery of rabbits with hindlimb ischemia, resulted in augmented development of collateral vessels (as documented by serial angiograms, increased capillary density, and increased capillary/myocyte ratio) (108). Consequent amelioration of the hemodynamic deficit in the ischemic limb was documented by improvement in the calf blood pressure ratio (ischemic/normal limb) to  $0.70 \pm 0.08$  in the VEGF-transfected group versus  $0.50 \pm 0.18$  in controls ( $P < 0.05$ ). These findings suggest that site-specific arterial gene transfer of plasmid-VEGF<sub>165</sub> may achieve physiologically meaningful therapeutic modulation of vascular insufficiency (109). In a similar study in the rabbit ischemic hindlimb model, VEGF-C (VEGF2) was administered as a



naked plasmid DNA (pcVEGF-C; 500  $\mu\text{g}$ ) from the polymer coating of an angioplasty balloon or as recombinant human protein (rhVEGF-C; 500  $\mu\text{g}$ ) by direct intra-arterial infusion.

Physiological and anatomical assessments of angiogenesis 30 days later showed evidence of therapeutic angiogenesis for both pcVEGF-C and rhVEGF-C. Hind-limb blood pressure ratio (ischemic/normal) increased to  $0.83 \pm 0.03$  after pcVEGF-C versus  $0.59 \pm 0.04$  ( $P = 0.005$ ) in plasmid controls, and to  $0.76 \pm 0.04$  after rhVEGF-C versus  $0.58 \pm 0.03$  ( $P = 0.01$ ) in control rabbits that received rabbit serum albumin. Doppler-derived iliac flow reserve was higher for the pcVEGF-C and rhVEGF-C rabbits than for controls. Neovascularity, documented by angiography in vivo and by capillary density, showed enhanced angiographic collaterals and capillary density with pcVEGF-C and rhVEGF-C relative to controls.

In summary, preclinical studies using both plasmid and adenoviral-based gene transfer of FGFs and VEGFs demonstrated functionally significant angiogenesis in various in vitro and in vivo models. These encouraging results have prompted clinical investigations of these gene-transfer strategies in patients with ischemic heart disease who are not candidates for percutaneous coronary intervention or coronary artery bypass surgery. These trials should be interpreted cautiously because of their uncontrolled, open-label design and a significant placebo effect seen in patients with end-stage coronary artery disease (101).

A phase I trial of plasmid-VEGF<sub>165</sub> delivered via direct intramyocardial injections (using open thoracotomy) was performed in patients with severe coronary artery disease who are not candidates for standard revascularization strategies (110). The first five patients were treated with naked plasmid DNA encoding VEGF<sub>165</sub>. Injections caused no changes in heart rate and blood pressure, and ventricular arrhythmias were limited to single unifocal premature beats during injection. Postoperative cardiac output fell transiently but increased within 24 h (preanesthesia:  $4.8 \pm 0.4$  L/min, postanesthesia:  $4.1 \pm 0.3$  L/min, 24 h postoperative:  $6.3 \pm 0.8$ ). All patients had significant reduction in angina (nitroglycerin use:  $53.9 \pm 10.0$  tablets per week at baseline and  $9.8 \pm 6.9$  tablets per week post-treatment). Reduced ischemia was observed, using dobutamine single photon emission computed tomography (SPECT)-sestamibi imaging in all patients. Coronary angiography showed improved Rentrop score (collateralization score) in 5 of 5 patients.

Twenty patients were subsequently enrolled in the phase I study (111). Plasma VEGF protein level increased from  $30.6 \pm 4.1$  pg/mL pretreatment to  $73.7 \pm 10.1$  pg/mL 14 days post-treatment ( $P = 0.0002$ ) and returned to baseline by day 90. However, it is uncertain whether this was due to the surgical trauma (open thoracotomy and intramyocardial injection of as much as 1 ml per injection). All 16 patients followed to day 90 reported a reduction in angina. A reduction in ischemic defects on SPECT-sestamibi scans was reported in 13 of 17 patients at 60 days. Angiographic evidence of improved collateral filling of at least one occluded vessel was observed in all patients evaluated at day 60.

Another phase I study used an E1(-)E3(-) adenovirus (Ad) gene-transfer vector expressing human VEGF<sub>121</sub> cDNA, administered by direct intramyocardial injection using open thoracotomy (112). Twenty-one patients were enrolled in the study and underwent intramyocardial injection of Ad-VEGF<sub>121</sub> as an adjunct to conventional coronary artery bypass grafting (group A,  $n = 15$ ) or as sole therapy via a thoracotomy (group B,  $n = 6$ ). There was no evidence of systemic or cardiac-related adverse events related to vector administration. In both groups, coronary angiography and stress-sestamibi scan assessment of wall motion 30 days after therapy suggested improvement in the area of vector administration and improvement in angina class. In group B, in which gene transfer was the sole therapy, treadmill exercise assessment suggested improvement in most individuals. A placebo-controlled study using catheter-based intramyocardial delivery is planned.

Although this review focuses on ischemic heart disease, it is important to briefly mention clinical angiogenesis studies that have targeted the peripheral vasculature in patients with intermittent claudication and critical limb ischemia (82, 113). Intramuscular injection of plasmid-VEGF<sub>165</sub> was performed in 62 patients with critical limb ischemia and 28 patients with intermittent claudication (113). Preliminary results from this study were reported to be promising; however, its open-label and uncontrolled design precludes any conclusions regarding efficacy (82, 113). Lower-extremity edema was observed in 31 of 90 patients (34%), possibly related to increased vascular permeability with VEGF<sub>165</sub>, otherwise known as vascular permeability factor (VPF).

The clinical results to date regarding gene transfer of angiogenic agents in ischemic heart disease have been limited with mostly uncontrolled studies showing preliminary evidence of efficacy. The viability of this treatment strategy will be determined by phase II and III, randomized double-blind, placebo-controlled studies of gene transfer of angiogenic agents to the heart of patients with ischemic heart disease that is not amenable to standard revascularization strategies. The availability of more advanced vector technology will improve the safety and efficacy profiles of these vectors, making gene transfer for therapeutic angiogenesis a reality.

Furthermore, it is important to emphasize the potential for these therapeutic agents and gene transfer vectors to result in pathologic angiogenesis (as discussed previously), as well as the potential for other serious adverse events including death, severe inflammatory reactions, dangerous ventricular tachyarrhythmias, and other unforeseen side effects. This is illustrated by the recent, tragic, and highly publicized death of Jesse Gelsinger, an 18-year-old subject enrolled in a University of Pennsylvania gene transfer study (114), and the deaths of patients enrolled in myocardial gene transfer angiogenesis studies at St. Elizabeth hospital in Boston and Cornell University in New York. Although possibly related to the underlying disease, at least in some cases, these adverse events have underscored the need for stricter adherence to good clinical practice (GCP) guidelines when conducting clinical research and the need for well-designed and controlled studies.

In a recent editorial, Health and Human Services Secretary Donna Shalala identified four major problems that need to be addressed to ensure adequate protection of human subjects in clinical research in general. The problems are as follows:

1. Researchers may not be doing enough to ensure that subjects fully understand all the potential risks and benefits of a clinical trial. Full disclosure is a necessary precondition to free choice. Accordingly, subjects who do not understand the potential risks of a trial cannot be said to have chosen freely to face those risks.
2. Researchers are not adhering to standards of good clinical practice. The FDA has identified cases in which researchers failed to disqualify subjects who did not meet the criteria for a study, failed to report adverse events as required, failed to ensure that a protocol was followed, and failed to ensure that study staff had adequate training.
3. Institutional review boards (IRB), the key element of the system to protect research subjects, have excessive workloads and inadequate resources.
4. Potential conflicts of interest and ethical dilemmas arise because academic researchers serve as both investigators and sponsors or patent holders of products. The researcher's self-interest may subtly influence his or her scientific judgment (114, 115).

These problems have to be resolved to ensure ethical conduct of research.

In addition to the protection of study subjects, all future studies of therapeutic angiogenesis should consider the following issues:

1. The placebo effect is extremely powerful in patients with end-stage cardiac disease.
2. Randomized clinical trials are powerful tools for eliminating confounding variables but cannot reduce investigator bias or the placebo effect.
3. Patient and investigator blinding would eliminate the placebo effect and investigator bias, effectively reduce crossover (which dilutes treatment effect), and minimize differences in other aspects of medical care.
4. Although blinding may be perceived as unethical, this perception may be overcome by thoughtful study design and investigators. In addition, it is probably more ethical to enroll a few patients in a placebo-controlled, well-designed and adequately powered study than to treat hundreds of patients with a treatment that is later shown to be ineffective.
5. No-option patients (end-stage coronary artery disease) may also exhibit a "physiological placebo" effect whereby ineffective treatment induces symptomatic relief paralleled by improved exercise tolerance, improved cardiac conditioning, and potential improved collateralization (by repeated myocardial ischemia).

## CONCLUSION

Cardiovascular gene transfer (the term gene therapy should be used only when we have data concerning the therapeutic potential of an agent) shows great promise in inducing angiogenesis, reducing restenosis, preventing graft failure after CABG, reducing cholesterol and preventing coronary atherosclerosis, and preventing coronary thrombosis and plaque rupture. However, the promise can be fulfilled only with the aid of significant advances in vector technology and delivery modalities, and most important, a better understanding of the biology of these processes. In addition, investigators must refrain from reporting anecdotal and uncontrolled study results. Such reports set the stage for unrealistic expectations for these treatment strategies, so that even modest successes might be viewed as failures. It is unlikely that the gene-transfer vectors of today will be the therapeutic agents of tomorrow; rather, continued advances will give us the ideal vector, molecule, and target, with the option to regulate its expression. Within its boundaries, gene transfer will open the door to a revolutionary treatment strategy that promises to benefit all patients with coronary artery disease.

## ACKNOWLEDGMENT

This work was supported in part by NIH grants MO1-RR01032 (RJL), HL 63609 and 56993 (MS), and HL 46716.

**Visit the Annual Reviews home page at [www.AnnualReviews.org](http://www.AnnualReviews.org)**

## LITERATURE CITED

1. American Heart Association. 2000. [www.americanheart.org](http://www.americanheart.org)
- 1a. Kilaru PK, Kelly RF, Calvin JE, Parrillo JE. 2000. Utilization of coronary angiography and revascularization after acute myocardial infarction in men and women risk stratified by the American College of Cardiology/American Heart Association guidelines. *J. Am. Coll. Cardiol.* 35:974–79
2. Eagle KA, Guyton RA, Davidoff R, et al. 1999. ACC/AHA Guidelines for coronary artery bypass graft surgery: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (committee to revise the 1991 Guidelines for Coronary Artery Bypass Graft Surgery). American College of Cardiology/American Heart Association. *J. Am. Coll. Cardiol.* 34:1262–347
3. Budoff MJ. 2000. Electron beam computed tomography: calcification and lipid lowering interventions. *Z. Kardiol.* 89:130–34
4. Busch T, Sirbu H, Aleksic I, et al. 2000. Importance of cardiovascular interventions before surgery for abdominal aortic aneurysms. *Cardiovasc. Surg.* 8:18–21
5. Mooney A. 1999. Treating patients with hypertriglyceridaemia saves lives: triglyceride revisited. *Curr. Med. Res. Opin.* 15:65–77
6. Tavazzi L. 1999. Clinical epidemiology of acute myocardial infarction. *Am. Heart J.* 138:48–54
7. Yutani C, Imakita M, Ishibashi-Ueda H, et al. 1999. Coronary atherosclerosis and

- interventions: pathological sequences and restenosis. *Pathol. Int.* 49:273–90
8. McPherson R. 1999. Controversies in primary prevention among hyperlipidemic patients. *Can. J. Cardiol.* 15(Suppl. B):16–9B
  9. Jones EL, Craver JM, Guyton RA, et al. 1983. Importance of complete revascularization in performance of the coronary bypass operation. *Am. J. Cardiol.* 51:7–12
  10. McNeer JF, Conley MJ, Starmer CF, et al. 1974. Complete and incomplete revascularization at aortocoronary bypass surgery: experience with 392 consecutive patients. *Am. Heart J.* 88:176–82
  11. Bauters C. 1997. Growth factors as a potential new treatment for ischemic heart disease. *Clin. Cardiol.* 20(II):52–57(Spec. issue)
  12. Folkman J. 1998. Angiogenic therapy of the human heart. *Circulation* 97:628–29
  13. Frazier OH, Kadipasaoglu KA, Cooley DA. 1998. Transmyocardial laser revascularization. Does it have a role in the treatment of ischemic heart disease? *Tex. Heart Inst. J.* 25:24–29
  14. Hussain FM, Heilbron M Jr. 1997. A review of the literature: transmyocardial laser revascularization. *J. Clin. Laser Med. Surg.* 15:57–63
  15. Sellke FW, Laham RJ, Edelman ER, et al. 1998. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. *Ann. Thorac. Surg.* 65:1540–44
  16. Sawamura D, Meng X, Ina S, et al. 2000. Expression vector with DNA of bovine papilloma virus 1 for keratinocyte gene therapy. *J. Dermatol. Sci.* 23:111–16
  17. Phillips MI. 2000. Somatic gene therapy for hypertension. *Braz. J. Med. Biol. Res.* 33:715–21
  18. Princen F, Lechanteur C, Lopez M, et al. 2000. Similar efficiency of DNA-liposome complexes and retrovirus-producing cells for HSV-tk suicide gene therapy of peritoneal carcinomatosis. *J. Drug Target* 8: 79–89
  19. Natsume A, Mizuno M, Ryuke Y, Yoshida J. 2000. Cationic liposome conjugation to recombinant adenoviral vector reduces viral antigenicity. *Jpn. J. Cancer Res.* 91:363–67
  20. DeBruyne LA, Li K, Bishop DK, Bromberg JS. 2000. Gene transfer of virally encoded chemokine antagonists vMIP-II and MC148 prolongs cardiac allograft survival and inhibits donor-specific immunity. *Gene Ther.* 7:575–82
  21. Ferreira GN, Cabral JM, Prazeres DM. 2000. Anion exchange purification of plasmid DNA using expanded bed adsorption. *Bioseparation* 9:1–6
  22. MacColl GS, Novo FJ, Marshall NJ, et al. 2000. Optimisation of growth hormone production by muscle cells using plasmid DNA. *J. Endocrinol.* 165:329–36
  23. Nishikawa M, Yamauchi M, Morimoto K, et al. 2000. Hepatocyte-targeted in vivo gene expression by intravenous injection of plasmid DNA complexed with synthetic multi-functional gene delivery system. *Gene Ther.* 7:548–55
  24. Shangquan T, Cabral-Lilly D, Purandare U, et al. 2000. A novel N-acyl phosphatidylethanolamine-containing delivery vehicle for spermine-condensed plasmid DNA. *Gene Ther.* 7:769–83
  25. Atienza C Jr, Elliott MJ, Dong YB, et al. 2000. Adenovirus-mediated E2F-1 gene transfer induces an apoptotic response in human gastric carcinoma cells that is enhanced by cyclin dependent kinase inhibitors. *Int. J. Mol. Med.* 6:55–63
  26. Bilbao R, Gerolami R, Bralet MP, et al. 2000. Transduction efficacy, antitumoral effect, and toxicity of adenovirus-mediated herpes simplex virus thymidine kinase/ganciclovir therapy of hepatocellular carcinoma: the woodchuck animal model. *Cancer Gene Ther.* 7:657–62
  27. Chen P, Kovesdi I, Bruder JT. 2000. Effective repeat administration with adenovirus

- vectors to the muscle. *Gene Ther.* 7:587–95
28. Lee EJ, Thimmapaya B, Jameson JL. 2000. Stereotactic injection of adenoviral vectors that target gene expression to specific pituitary cell types: implications for gene therapy. *Neurosurgery* 46:1461–68; discussion 168–69
  29. Smith P, Shuler FD, Georgescu HI, et al. 2000. Genetic enhancement of matrix synthesis by articular chondrocytes: comparison of different growth factor genes in the presence and absence of interleukin-1. *Arthritis Rheum.* 43:1156–64
  30. Walter G, Barton ER, Sweeney HL. 2000. Noninvasive measurement of gene expression in skeletal muscle. *Proc. Natl. Acad. Sci. USA* 97:5151–55
  31. Zhang R, DeGroot LJ. 2000. Gene therapy of established medullary thyroid carcinoma with herpes simplex viral thymidine kinase in a rat tumor model: relationship of bystander effect and antitumor efficacy. *Thyroid* 10:313–19
  32. Kumar-Singh R, Yamashita CK, Tran K, Farber DB. 2000. Construction of encapsidated (guttled) adenovirus minichromosomes and their application to rescue of photoreceptor degeneration. *Methods Enzymol.* 316:724–43
  33. Hartigan-O'Connor D, Chamberlain JS. 2000. Developments in gene therapy for muscular dystrophy. *Microsc. Res. Tech.* 48:223–38
  34. Hartigan-O'Connor D, Amalfitano A, Chamberlain JS. 1999. Improved production of gutted adenovirus in cells expressing adenovirus preterminal protein and DNA polymerase. *J. Virol.* 73:7835–41
  35. Duthel N, Shi F, Dupressoir T, Linden RM. 2000. Adeno-associated virus site-specifically integrates into a muscle-specific DNA region. *Proc. Natl. Acad. Sci. USA* 97:4862–66
  36. Drittanti L, Rivet C, Manceau P, et al. 2000. High throughput production, screening and analysis of adeno-associated viral vectors. *Gene Ther.* 7:924–29
  37. Hudde T, Rayner SA, De Alwis M, et al. 2000. Adeno-associated and herpes simplex viruses as vectors for gene transfer to the corneal endothelium. *Cornea* 19:369–73
  38. Debelak D, Fisher J, Iuliano S, et al. 2000. Cation-exchange high-performance liquid chromatography of recombinant adeno-associated virus type 2. *J. Chromatogr. B Biomed. Sci. Appl.* 740:195–202
  39. Sun L, Li J, Xiao X. 2000. Overcoming adeno-associated virus vector size limitation through viral DNA heterodimerization. *Nat. Med.* 6:599–602
  40. Duan D, Yue Y, Yan Z, Engelhardt JF. 2000. A new dual-vector approach to enhance recombinant adeno-associated virus-mediated gene expression through intermolecular cis activation. *Nat. Med.* 6:595–98
  41. Nakai H, Storm TA, Kay MA. 2000. Increasing the size of rAAV-mediated expression cassettes in vivo by intermolecular joining of two complementary vectors. *Nat. Biotechnol.* 18:527–32
  42. Rudich SM, Zhou S, Srivastava R, et al. 2000. Dose response to a single intramuscular injection of recombinant adeno-associated virus-erythropoietin in monkeys. *J. Surg. Res.* 90:102–8
  43. Bohl D, Bosch A, Cardona A, et al. 2000. Improvement of erythropoiesis in beta-thalassemic mice by continuous erythropoietin delivery from muscle. *Blood* 95:2793–98
  44. Hirata RK, Russell DW. 2000. Design and packaging of adeno-associated virus gene targeting vectors. *J. Virol.* 74:4612–20
  45. Costello E, Munoz M, Buetti E, et al. 2000. Gene transfer into stimulated and unstimulated T lymphocytes by HIV-1-derived lentiviral vectors. *Gene Ther.* 7:596–604
  46. Ng YY, Veenhuizen P, Lokhorst H, Staal FJ. 2000. Frpe-1 and Frpe-3: two different recombinant retroviruses encoding the same human marker gene. *Cancer Gene Ther.* 7:624–28

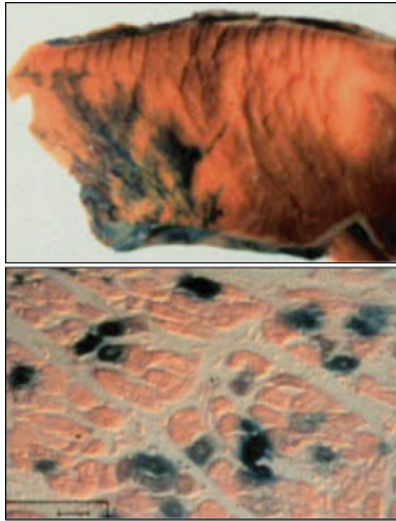
47. Palu G, Parolin C, Takeuchi Y, Pizzato M. 2000. Progress with retroviral gene vectors. *Rev. Med. Virol.* 10:185–202
48. Parveen Z, Krupetsky A, Engelstadter M, et al. 2000. Spleen necrosis virus-derived C-type retroviral vectors for gene transfer to quiescent cells. *Nat. Biotechnol.* 18:623–29
49. Solaiman F, Zink MA, Xu G, et al. 2000. Modular retro-vectors for transgenic and therapeutic use. *Mol. Reprod. Dev.* 56:309–15
50. Marshall E. 2000. Improving gene therapy's tool kit. *Science* 288:953
51. Follenzi A, Ailles LE, Bakovic S, et al. 2000. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. *Nat Genet.* 25:217–22
52. Johnson LG, Olsen JC, Naldini L, Boucher RC. 2000. Pseudotyped human lentiviral vector-mediated gene transfer to airway epithelia in vivo. *Gene Ther.* 7:568–74
53. Dupont F, Avalosse B, Karim A, et al. 2000. Tumor-selective gene transduction and cell killing with an oncotropic autonomous parvovirus-based vector. *Gene Ther.* 7:790–96
54. Karpoff HM, Kooby D, D'Angelica M, et al. 2000. Efficient cotransduction of tumors by multiple herpes simplex vectors: implications for tumor vaccine production. *Cancer Gene Ther.* 7:581–88
55. Pineau P, Meddeb M, Raselli R, et al. 2000. Effect of TT virus infection on hepatocellular carcinoma development: results of a Euro-Asian survey. *J. Infect. Dis.* 181:1138–42
56. Stone D, David A, Bolognani F, et al. 2000. Viral vectors for gene delivery and gene therapy within the endocrine system. *J. Endocrinol.* 164:103–18
57. Candotti F, Agbaria R, Mullen CA, et al. 2000. Use of a herpes thymidine kinase/neomycin phosphotransferase chimeric gene for metabolic suicide gene transfer. *Cancer Gene Ther.* 7:574–80
58. Hoshi M, Harada A, Kawase T, et al. 2000. Antitumoral effects of defective herpes simplex virus-mediated transfer of tissue inhibitor of metalloproteinases-2 gene in malignant glioma U87 in vitro: consequences for anti-cancer gene therapy. *Cancer Gene Ther.* 7:799–805
59. Cioffi L, Sturtz FG, Wittmer S, et al. 1999. A novel endothelial cell-based gene therapy platform for the in vivo delivery of apolipoprotein E. *Gene Ther.* 6:1153–59
60. Powell C, Shansky J, Del Tatto M, et al. 1999. Tissue-engineered human bioartificial muscles expressing a foreign recombinant protein for gene therapy. *Hum. Gene Ther.* 10:565–77
61. Rinsch C, Regulier E, Deglon N, et al. 1997. A gene therapy approach to regulated delivery of erythropoietin as a function of oxygen tension. *Hum. Gene Ther.* 8:1881–89
62. Su L, Lee R, Bonyhadi M, et al. 1997. Hematopoietic stem cell-based gene therapy for acquired immunodeficiency syndrome: efficient transduction and expression of RevM10 in myeloid cells in vivo and in vitro. *Blood* 89:2283–90
63. Zhang J, Russell SJ. 1996. Vectors for cancer gene therapy. *Cancer Metastasis Rev.* 15:385–401
64. Nam M, Johnston P, Lal B, et al. 1996. Endothelial cell-based cytokine gene delivery inhibits 9L glioma growth in vivo. *Brain Res.* 731:161–70
65. Wei Y, Quertermous T, Wagner TE. 1995. Directed endothelial differentiation of cultured embryonic yolk sac cells in vivo provides a novel cell-based system for gene therapy. *Stem Cells* 13:541–47
66. Nagy JA, Shockley TR, Masse EM, et al. 1995. Systemic delivery of a recombinant protein by genetically modified mesothelial cells reseeded on the parietal peritoneal surface. *Gene Ther.* 2:402–10
67. Rosenthal FM, Fruh R, Henschler R, et al. 1994. Cytokine therapy with gene-transfected cells: single injection of

- irradiated granulocyte-macrophage colony-stimulating factor-transduced cells accelerates hematopoietic recovery after cytotoxic chemotherapy in mice. *Blood* 84:2960–65
68. Simons M, Laham R. 1999. Therapeutic angiogenesis in myocardial ischemia. In *Angiogenesis and Cardiovascular Disease*, ed. JA Ware, M Simons, pp. 289–320. New York: Oxford Univ. Press
69. Laham RJ, Rezaee M, Post M, et al. 1999. Intracoronary and intravenous administration of basic fibroblast growth factor: myocardial and tissue distribution. *Drug Metab. Dispos.* 27:821–26
70. Ware JA, Simons M. 1997. Angiogenesis in ischemic heart disease. *Nat. Med.* 3:158–64
71. Simons M, Ware JA. 1996. Food for starving hearts. *Nat. Med.* 2:519–20
72. Iosseliani DG, Koval AN, Bhattacharya PK. 1994. Myocardial perfusion in new-onset angina patients with single vessel disease. *Int. J. Cardiol. Imaging* 10:155–59
73. Juilliere Y, Marie PY, Danchin N, et al. 1991. Evolution of myocardial ischemia and left ventricular function in patients with angina pectoris without myocardial infarction and total occlusion of the left anterior descending coronary artery and collaterals from other coronary arteries. *Am. J. Cardiol.* 68:7–12
74. Kinn JW, Altman JD, Chang MW, Bache RJ. 1996. Vasomotor responses of newly developed coronary collateral vessels. *Am. J. Physiol.* 271:H490–97
75. Nishigami K, Ando M, Hayasaki K. 1995. Effects of antecedent anginal episodes and coronary artery stenosis on left ventricular function during coronary occlusion. *Am. Heart J.* 130:244–47
76. Sabri MN, DiSciascio G, Cowley MJ, et al. 1991. Coronary collateral recruitment: functional significance and relation to rate of vessel closure. *Am. Heart J.* 121:876–80
77. Sasayama S, Fujita M. 1992. Recent insights into coronary collateral circulation. *Circulation* 85:1197–204
78. Stone DA, Corretti MC, Hawke MW, et al. 1995. The influence of angiographically demonstrated coronary collaterals on the results of stress echocardiography. *Clin. Cardiol.* 18:205–8
79. Schaper W, Ito W. 1996. Molecular mechanisms of collateral vessel growth. *Circ. Res.* 79:911–19
80. Laham R, Rezaee M, Post M, et al. 2000. Intrapericardial delivery of fibroblast growth factor-2 induces neovascularization in a porcine model of chronic myocardial ischemia. *J. Pharmacol. Exp. Ther.* In press
81. Isner JM, Walsh K, Symes J, et al. 1995. Arterial gene therapy for therapeutic angiogenesis in patients with peripheral artery disease. *Circulation* 91:2687–92
82. Isner JM, Pieczek A, Schainfeld R, et al. 1996. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF<sub>165</sub> in a patient with ischaemic limb. *Lancet* 348:370–74
83. Mack CA, Patel SR, Schwarz EA, et al. 1998. Biologic bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. *J. Thorac. Cardiovasc. Surg.* 115:168–76; discussion 176–77
84. Magovern CJ, Mack CA, Zhang J, et al. 1996. Direct in vivo gene transfer to canine myocardium using a replication-deficient adenovirus vector. *Ann. Thorac. Surg.* 62:425–33; discussion 433–34
85. Giordano FJ, Ping P, McKirnan MD, et al. 1996. Intracoronary gene transfer of fibroblast growth factor-5 increases blood flow and contractile function in an ischemic region of the heart. *Nat. Med.* 2:534–39
86. Muhlhauser J, Pili R, Merrill MJ, et al. 1995. In vivo angiogenesis induced by recombinant adenovirus vectors coding either for secreted or nonsecreted forms of



- acidic fibroblast growth factor. *Hum. Gene Ther.* 6:1457–65
87. Buschmann I, Schaper W. 2000. The pathophysiology of the collateral circulation (arteriogenesis). *J. Pathol.* 190:338–42
88. Carmeliet P, Collen D. 2000. Transgenic mouse models in angiogenesis and cardiovascular disease. *J. Pathol.* 190:387–405
89. Risau W, Flamme I. 1995. Vasculogenesis. *Ann. Rev. Cell. Dev. Biol.* 11:79–91
90. Asahara T, Masuda H, Takahashi T, et al. 1999. Bone marrow origin of endothelial progenitor cells responsible for post-natal vasculogenesis in physiological and pathological neovascularization. *Circ. Res.* 85:221–28
91. Folkman J, Shing Y. 1992. Angiogenesis. *J. Biol. Chem.* 267:10931–34
92. Li J, Brown LF, Hibberd MG, et al. 1996. VEGF, flk-1, and flt-1 expression in a rat myocardial infarction model of angiogenesis. *Am. J. Physiol.* 270:H1803–11
93. Arras M, Ito WD, Scholz D, et al. 1998. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J. Clin. Invest.* 101:40–50
94. Arras M, Strasser R, Mohri M, et al. 1998. Tumor necrosis factor- $\alpha$  is expressed by monocytes/macrophages following cardiac microembolization and is antagonized by cyclosporine. *Basic Res. Cardiol.* 93:97–107
95. Li J, Brown LF, Laham RJ, et al. 1997. Macrophage-dependent regulation of syndecan gene expression. *Circ. Res.* 81:785–96
96. Li J, Post M, Volk R, et al. 2000. PR39, a peptide regulator of angiogenesis. *Nat. Med.* 6:49–55
97. Jones MK, Wang H, Peskar BM, et al. 1999. Inhibition of angiogenesis by non-steroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. *Nat. Med.* 5:1418–23
98. Carmeliet P, Dor Y, Herbert JM, et al. 1998. Role of HIF-1 $\alpha$  in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394:485–90
99. Semenza GL. 2000. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J. Appl. Physiol.* 88:1474–80
100. Laham R, Sellke F, Edelman E, et al. 1999. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: results of a phase I randomized, double-blind, placebo-controlled trial. *Circulation* 100:1865–71
101. Henry T, Annex B, Azrin M, et al. 1999. Double blind placebo controlled trial of recombinant human vascular endothelial growth factor—the VIVA trial. *J. Am. Coll. Cardiol.* 33:384A
102. Laham R, Chronos N, Pike M, et al. 2000. Intracoronary basic fibroblast growth factor (FGF-2) in patients with severe ischemic heart disease: results of a phase I open label dose escalation study. *J. Am. Coll. Cardiol.* In press
103. Iyer NV, Kotch LE, Agani F, et al. 1998. Cellular and developmental control of O<sub>2</sub> homeostasis by hypoxia-inducible factor 1  $\alpha$ . *Genes Dev.* 12:149–62
104. Kotch LE, Iyer NV, Laughner E, Semenza GL. 1999. Defective vascularization of HIF-1 $\alpha$ -null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev. Biol.* 209:254–67
105. Yu AY, Shimoda LA, Iyer NV, et al. 1999. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 $\alpha$ . *J. Clin. Invest.* 103:691–96
106. Muhlhauser J, Merrill MJ, Pili R, et al. 1995. VEGF<sub>165</sub> expressed by a replication-deficient recombinant adenovirus vector induces angiogenesis in vivo. *Circ. Res.* 77:1077–86
107. Magovern CJ, Mack CA, Zhang J, et al. 1997. Regional angiogenesis induced in nonischemic tissue by an adenoviral

- vector expressing vascular endothelial growth factor. *Hum. Gene Ther.* 8:215–27
108. Takeshita S, Weir L, Chen D, et al. 1996. Therapeutic angiogenesis following arterial gene transfer of vascular endothelial growth factor in a rabbit model of hindlimb ischemia. *Biochem. Biophys. Res. Commun.* 227:628–35
109. Witzensichler B, Asahara T, Murohara T, et al. 1998. Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. *Am. J. Pathol.* 153:381–94
110. Losordo DW, Vale PR, Symes JF, et al. 1998. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF<sub>165</sub> as sole therapy for myocardial ischemia. *Circulation* 98:2800–4
111. Symes JF, Losordo DW, Vale PR, et al. 1999. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. *Ann. Thorac. Surg.* 68:830–36; discussion 836–37
112. Rosengart TK, Lee LY, Patel SR, et al. 1999. Angiogenesis gene therapy: phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF<sub>121</sub> cDNA to individuals with clinically significant severe coronary artery disease. *Circulation* 100:468–74
113. Baumgartner I, Rauh G, Pieczek A, et al. 2000. Lower-extremity edema associated with gene transfer of naked DNA encoding vascular endothelial growth factor. *Ann. Intern. Med.* 132:880–84
114. Shalala D. 2000. Protecting research subjects—what must be done. *N. Engl. J. Med.* 343:808–10
115. Angell M. 2000. Is academic medicine for sale? *N. Engl. J. Med.* 342:1516–18



**Figure 1** Intramyocardial injection of adenoviral-LacZ (cytomegalovirus promoter) using the Boston Scientific<sup>R</sup>/Scimed<sup>R</sup> catheter.  $10^{10}$  PFU viral particles were used per injection volume ( $50 \mu\text{l}$ ). LacZ was detected by incubation of sections with  $\beta$ -galactosidase, which stains cells expressing LacZ in blue. *Top*: Gross myocardial sections with injection of solution containing  $10^{10}$  PFU viral particles. *Bottom*: Histologic analysis of myocardial sections with injection of  $50 \mu\text{l}$  of solution (containing  $10^{10}$  PFU viral particles) using the Boston Scientific<sup>R</sup>/Scimed<sup>R</sup> catheter. Note transfected cells stained in blue. (Courtesy of Boston Scientific).