Assessment of Risks Associated With Cardiovascular Gene Therapy in Human Subjects

Jeffrey M. Isner, Peter R. Vale, James F. Symes, Douglas W. Losordo

Abstract—Clinical trials of cardiovascular gene therapy, whether using viral (53%) or nonviral (47%) vectors, have thus far disclosed no evidence indicative of inflammatory or other complications, including death, directly attributable to the vector used. Indeed, despite the fact that initial trials of cardiovascular gene therapy targeted patients with end-stage vascular disease, including critical limb ischemia and refractory myocardial ischemia, the mortality for patients enrolled in clinical trials of cardiovascular gene therapy reported to date compares favorably with mortality for similar groups of patients in contemporary controlled studies of medical or interventional therapies. The most common morbidity reported after cardiovascular gene transfer is lower extremity edema; in contrast to data involving genetically engineered mice, however, evidence of life- or limb-threatening edema has not been described in any patients, including patients after gene transfer for myocardial ischemia. Concerns regarding the potential for angiogenic cytokines to promote the progression of atherosclerosis are not supported by angiographic follow-up of patients with coronary or peripheral vascular disease. The levels and duration of gene expression investigated for therapeutic angiogenesis transfer have been unassociated with hemangioma formation. Likewise, there is little evidence from either preclinical or clinical studies to support the notion that the administration of angiogenic growth factors, per se, is sufficient to stimulate the growth of neoplasms. Patients enrolled in clinical studies of angiogenic cytokines, including patients with diabetes and a previous history of retinopathy, have disclosed no evidence to suggest that ocular pathology is a risk of angiogenic growth factor gene transfer. (Circ Res. 2001;89:389-400.)

Key Words: gene therapy ■ coronary artery disease ■ peripheral vascular disease

Early phase 1 trials of cardiovascular gene therapy have disclosed potentially favorable results. These include the following: limb salvage in patients recommended for lower extremity amputation; resolution of sensory deficits in patients with ischemic peripheral neuropathy; reduced angina, improved exercise tolerance, and improved myocardial perfusion in patients with coronary heart disease; and reduced failure of lower extremity bypass grafts. As phase 1 trials, however, these studies were principally designed to gather safety and toxicity data. Indeed, at this stage of study, the developing safety profile is clearly the principal determinant governing the extent to which such investigations may progress to phase 3 trials, designed to establish proof of efficacy. During the past 18 months, regulatory scrutiny regarding the risks of gene therapy in general, and cardiovascular gene therapy in particular, has been intensified.
to an unusual degree after the death of Jesse Gelsinger at the University of Pennsylvania.

Accordingly, this analysis will consider those data that have been publicly discussed and/or formally reported to provide some broad assessment of the risks thus far exposed by clinical application of cardiovascular gene transfer to human subjects.

Current Scope of Gene Therapy for Cardiovascular Disorders

Clinical applications of cardiovascular gene therapy began on December 7, 1994. Since that initial experiment, cardiovascular trials have increased in proportion to the rest of the field and become more diverse. Thus, although cardiovascular disorders have been the indication for only 9% of all gene transfer trials proposed to the National Institutes of Health (NIH) over the past decade, an increase in the number of cardiovascular proposals submitted over the past 3 years resulted by the year 2000 in 17% of all gene therapy trials addressing cardiovascular disorders (Figure 1). This increase in proposals for cardiovascular trials contrasts with the nearly 50% reduction in proposals for other disease states that occurred during the 1-year period after the Gelsinger death (C. Mickelson, PhD, oral communication, September 2000).

The increase in cardiovascular studies is the result of several factors. First, in common with other indications for gene therapy, the universe of cardiovascular disease encompasses multiple unmet needs. These include ischemic heart disease refractory to medical therapy and unsuitable for conventional revascularization, critical limb ischemia unsuitable for angioplasty or bypass surgery, coronary and peripheral arterial restenosis, ischemic and/or diabetic neuropathy, lymphedema, and advanced heart failure.

Second, in contrast to most applications of noncardiovascular gene therapy, the strategies proposed to address certain of these unmet needs are well matched for the current toolbox of gene therapy. Initial applications of gene therapy (Figure 2A) involved monogenic diseases, such as cystic fibrosis or muscular dystrophy, in which defunct gene expression from birth would lead in short order to a variety of adverse outcomes; gene therapy for these disorders was envisioned as lifelong gene replacement that would preclude pathological consequences. An implicit requirement for such a strategy is the availability of vectors that could provide robust gene expression in a relatively large population of target cells (or even tissues) for an extended, if not indefinite, period of time. Not surprisingly, most attempts to address such disorders with currently available, including viral, vectors failed. In contrast, initial proposals to apply gene transfer to cardiovascular disease identified disorders in which the requisite rise in the expression of a particular gene (eg, one encoding for an angiogenic growth factor) that was required in response to a given insult (eg, arterial occlusion) proved insufficient at some discrete point in an individual’s lifetime (eg, late adulthood) (B); transient gene replacement, sufficient to generate the appropriate biological response (eg, collateral vessel growth) might prove adequate to prevent the development of pathological consequences (eg, loss of limb or recurrent myocardial ischemia) (C and D).
even tissues) for an extended, if not indefinite, period of time. Not surprisingly, most attempts to address such disorders with currently available, including viral, vectors failed.12,13

In contrast, initial proposals11,14 to apply gene transfer to cardiovascular disease identified disorders in which the requisite rise in the expression of a particular gene (eg, one encoding for an angiogenic growth factor) that was required in response to a given insult (eg, arterial occlusion) proved insufficient at some discrete point in an individual’s lifetime (eg, late adulthood) (Figure 2B); transient gene replacement, sufficient to generate the appropriate biological response (eg, collateral vessel growth), might prove adequate to prevent the development of pathological consequences (eg, loss of limb or recurrent myocardial ischemia) (Figures 2C and 2D).

The third factor contributing to the increasing application of gene transfer to cardiovascular disorders is a corollary of the principle illustrated in Figure 2, namely, that because certain disorders might be successfully addressed by localized short-term gene expression, the complexity of vectors required might be simplified. Indeed, among the universe of clinical gene therapy trials, the cardiovascular portfolio is remarkable for the extent to which nonviral vectors are represented. Fully 47% of these trials use either naked plasmid DNA (39%) or liposome carriers (8%) (Figure 3A). This is in direct contrast to noncardiovascular trials, most of which use viral vectors, including adenovirus, adeno-associated virus, or retrovirus.

The increasing representation of cardiovascular disease among clinical investigations of gene therapy and the disproportionate use of nonviral vectors are neither unrelated nor accidental. This strategy runs counter to notions that prevailed for most of the first decade of gene therapy. During those years, it was suggested that the use of naked DNA was too inefficient to constitute an effective gene transfer strategy. In those years, most attention was focused on the use of viral vectors, intended to increase the magnitude and/or duration of gene expression. Indeed, a variety of approaches have been devised that would permit regulated gene expression15 to optimize safety and minimize the toxicity associated with protracted gene expression achievable with adeno-associated virus or lentiviral vectors.

However, preclinical studies carried out in our laboratory in 1993 to 1994 have suggested that genes encoding proteins that are naturally secreted from intact cells can achieve meaningful biological outcomes despite low transfection efficiency.16,17 To be explicit, transfer of genes that encode proteins that remain intracellular to achieve a biological effect (eg, the normal membrane channel gene that is defective in cystic fibrosis) must be delivered to a relatively large target population of cells to correct the underlying pathogenetic defect. In contrast, transfer of genes that encode proteins that are naturally secreted (eg, vascular endothelial growth factor [VEGF]) can achieve a biological effect by transfecting limited numbers of cells, provided that the transfected cells secrete substantial amounts of the gene product; the paracrine effect of the secreted gene product may then modulate the bioactivity of a large number of target (eg, endothelial) cells. Analysis of transgenes selected for cardiovascular trials indicates a predominance of vectors encoding angiogenic growth factors with a native or ligated signal sequence permitting active secretion (Figure 3B).

The disproportionate representation of nonviral vectors among cardiovascular protocols likely also reflects the growing notion that for cardiovascular gene therapy in particular, the efficiency of naked DNA gene transfer can be optimized significantly, perhaps to the point at which it is equivalent to adenoviral gene transfer, by modifications in backbone vector18,19 and/or the mode of physical delivery20–23 to achieve meaningful clinical results while minimizing safety concerns. Indeed, very recent data24 have demonstrated that adjunctive use of ultrasound exposure increases the efficiency of naked DNA gene transfer to the point that meaningful biological responses can be achieved with even nonsecreted gene products, such as p53.

Moreover, the need for long-term gene expression, which may be a prerequisite for certain types of inherited genetic defects, appears not to be a requirement for gene transfer strategies designed to promote neovascularization or inhibit restenosis. For the former, 2 to 3 weeks of gene expression appears to be sufficient to promote neovascularization; persistent blood flow,25 rather than persistent gene expression, constitutes the principal determinant of subsequent vascular maturation and durability. For restenosis, gene transfer of endothelial cell mitogens may confer a long-term benefit by sustained downregulation of subendothelial proliferative processes after expedited reendothelialization,14 whereas critically timed, albeit transient, application of antiproliferative strategies may result in long-term protection from bypass graft anastomotic narrowing.10

Thus, regardless of the transgene used, the potential applicability of nonviral vectors represents an important factor that...
may potentially enhance the safety profile of cardiovascular applications of gene therapy. However, it must be acknowledged that the risks of viral gene transfer, alleged to be responsible for the Gelsinger death, cannot necessarily be extrapolated to all strategies of gene transfer. Indeed, the Gelsinger death involved direct intrahepatic arterial infusion of a dose of adenoviral vector ($3.2 \times 10^{13}$ viral particles) that was 3 logs in excess of the largest dose of adenoviral vector to be administered to date in patients with cardiovascular disease (typically $10^7$ to $10^{10}$ viral particles). Reported data from cardiovascular trials of gene therapy using viral vectors (all adenovirus) have established that neutralizing antibodies directed against adenovirus 5 are increased in most or all patients. Systemic circulation of viral particles has not been detected after intramuscular adenoviral gene transfer but is common after intracoronary administration.

Although certain preclinical studies have been interpreted to suggest that inflammation provoked by the use of viral vectors increases the risk of gene transfer, other preclinical studies have failed to elicit significant evidence of an inflammatory reaction. Moreover, even when inflammation is recognized to accompany certain cardiovascular applications of gene transfer, it cannot necessarily be inferred that such pathology at an anatomic level will equate to pathologic clinical consequences. Indeed, Rosengart et al have reported no increase in serum creatine phosphokinase or other findings indicative of myocarditis in patients receiving adenoviral (Ad)/VEGF121 gene transfer as sole therapy. Fever has been occasionally observed after intra-arterial gene transfer of Ad/VEGF165 and was reported in three (5%) of the patients receiving intracoronary Ad/fibroblast growth factor (FGF)-4. Abnormal liver function tests were documented in only two (3.2%) of the patients undergoing Ad/FGF-4 gene transfer. “Occasional” patients after Ad/VEGF165 and in an unspecified number of patients after Ad/VEGF121, although no dose-response relationship could be shown in the latter. In toto, no serious adverse events attributable to viral vectors have been observed to date in clinical trials of adenoviral gene transfer involving $\approx 150$ patients receiving VEGF165, VEGF121, FGF-4, or hypoxia inducible factor (HIF)-1α/etoposide (VP-16).

**Mortality**

Initial clinical trials of gene therapy have typically been designed to address unmet needs among medical disorders. A standard principle for the investigation of any novel therapy is that the risk/benefit ratio may be minimized by applying a therapy with unknown risks to patients who are so severely ill that they have little to lose. This has been the case for most early clinical trials of gene therapy in general and cardiovascular gene therapy in particular. Thus, the first trial for cardiovascular disorders identified patients with critical limb ischemia who had exhausted all conventional options for revascularization, including angioplasty and bypass surgery. Psychological testing of such patients has disclosed quality-of-life indices similar to those of patients with cancer in the terminal phase of their illness. Because there is no medical therapy available for these patients, the likelihood of limb loss is high, and 5-year survival is poor. After establishing proof of concept in this population, these studies were extended to patients with myocardial ischemia who had similarly exhausted conventional options for revascularization and proved refractory to available medical therapy. Because myocardial perfusion is severely impaired in such patients, their activities are severely limited by exertion-induced angina, and they are at risk of recurrent infarction and/or death.

Although this approach works to preclude the development of unknown, including lethal, risks in healthy subjects with a reasonable life expectancy, a potential liability is the problem of sorting out adverse events that are due to the underlying disease versus the transgene or vector used. In the case of trials involving patients with critical limb ischemia, for example, it must be recognized that the 2-year mortality for such patients, which is primarily due to comorbid conditions, including coronary artery disease, has been reported to be as high as 32%. Risks associated with underlying disease in the case of myocardial ischemia include progression of atherosclerosis not only in the native coronary circulation but also in the remaining (often single) bypass grafts that constitute source vessels feeding whatever collateral circulation develops in response to therapeutic angiogenesis in the ischemic myocardium. This is compounded by the nonrandomized design and small sample size typical of phase 1 trials, intended to provide information regarding potential toxicities associated with various routes, regimens, and doses of therapy.

Despite this complex background, including the grim prognosis of patients with critical limb ischemia and the refractory clinical status of patients with myocardial ischemia, available safety data concerning the most adverse outcome, mortality (Table 1), in these earliest trials of cardiovascular gene therapy is encouraging. Among 100 patients with critical limb ischemia undergoing VEGF gene transfer at our institution, there have been 9 deaths in 7 years, reflecting a cumulative mortality of 9.0% that compares favorably with any published mortality figures for such patients. None were procedural deaths, nor were any deaths attributable to the transgene used. Similarly, among 85 patients with myocardial ischemia participating in four protocols using naked DNA VEGF gene transfer, there have been a total of three deaths, reflecting a cumulative mortality of 3.5%, at up to 33 months of follow-up (Figure 4). Among 97 patients followed for 1 to 3 years after myocardial gene transfer of adenovirus encoding either FGF-2 or VEGF121, five deaths have been reported, reflecting a cumulative mortality of 5.2%. These outcomes compare favorably with an average 1-year mortality of 11% to 13% for a similar group of almost 1000 patients receiving laser myocardial revascularization, which is an approved and coded procedure now being used in routine clinical practice, or continued medical therapy in five contemporary controlled studies.

**Morbidity**

**Accelerated Atherosclerosis**

Classic studies by Kwon et al, Williams et al, Barger et al, and others have established evidence that development
of atherosclerotic plaque is associated with proliferation of the vasa vasorum. Williams et al further showed that plaque regression, accomplished by withdrawal of a hypercholesterolemic diet, was associated with loss of vasa vasorum and a profound reduction in blood flow through the vasa to the coronary intima and media. The development of potent, apparently endothelial cell–specific, inhibitors of angiogenesis allowed Moulton et al to measure the impact of restricted neovascularization on plaque development. Moulton et al observed that when hypercholesterolemic mice were treated for 16 weeks with endostatin or TNP-470, the median plaque area was reduced by 85% and 70%, respectively. Moulton’s study, together with several other studies in particular, raised concern regarding the potential for VEGF and other proangiogenic therapies to promote atherosclerosis.

There is now a plethora of in vivo data (including human subjects) that refute the notion that accelerated atherosclerosis is a likely consequence of administering angiogenic cytokines (Table 2).

TABLE 1. Patient Deaths After Cardiovascular Gene Transfer

<table>
<thead>
<tr>
<th>Patient (Indication)</th>
<th>Sex</th>
<th>Age at Death, y</th>
<th>Vector/Transgene</th>
<th>Mode of Delivery</th>
<th>Interval From Gene Transfer to Date of Death</th>
<th>Nature of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (CLI) M 56</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IA 3 y</td>
<td>?MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (CLI) M 61</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IA 11 mo</td>
<td>?MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (CLI) M 84</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IA 3½ y</td>
<td>Cardiac arrest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (CLI) M 73</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IA 2 y</td>
<td>Cardiac arrest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (CLI) M 88</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IA 2 mo</td>
<td>Severe coronary atherosclerosis and interstitial myocardial fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (CLI) F 77</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IM 7 mo</td>
<td>Suicide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (CLI) F 65</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IM 5 mo</td>
<td>Suicide</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8 (CLI) F 57</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IM 13 mo</td>
<td>Severe accidental head trauma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (CLI) M 76</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; IA 58 mo</td>
<td>Cardiac arrest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (restenosis) M 83</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt; IA 70 mo</td>
<td>Lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 (restenosis) M 73</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IM 16 mo</td>
<td>Respiratory insufficiency, renal failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 (restenosis) F 71</td>
<td>NV/VEGF&lt;sub&gt;165&lt;/sub&gt; IM 4 mo</td>
<td>CAD/cardiogenic shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (restenosis) M 59</td>
<td>NV/VEGF&lt;sub&gt;2&lt;/sub&gt; IM 1 d</td>
<td>Sudden death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 (restenosis) M 68</td>
<td>Ad/FGF-4 IC 145 d</td>
<td>Colon cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (restenosis) M 61</td>
<td>Ad/VEGF&lt;sub&gt;121&lt;/sub&gt; IM 40 d</td>
<td>MI, pneumonia, lung abscess</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 (restenosis) F 85</td>
<td>Ad/VEGF&lt;sub&gt;121&lt;/sub&gt; IM 5 d</td>
<td>Ileocolic necrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 (restenosis) M 61</td>
<td>Ad/VEGF&lt;sub&gt;121&lt;/sub&gt; IM 3 mo</td>
<td>Sudden death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 (restenosis) F 57</td>
<td>Ad/VEGF&lt;sub&gt;121&lt;/sub&gt; IM 5 mo</td>
<td>Suicide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 (restenosis) M 61</td>
<td>Ad/VEGF&lt;sub&gt;121&lt;/sub&gt; IM 3 mo</td>
<td>Suicide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (restenosis) M 61</td>
<td>Ad/VEGF&lt;sub&gt;121&lt;/sub&gt; IM 3 mo</td>
<td>Suicide</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

CLI indicates critical limb ischemia; CAD, coronary artery disease; M, male; F, female; NR, not reported; NV, nonviral; IA, intra-arterial; IM, intramuscular; IMyo(op), intramyocardial (intraoperative); IC, intracoronary; and MI, myocardial infarction.

Myocardial Gene Transfer Mortality

Figure 4. Cumulative mortality among 85 patients (pts) with myocardial ischemia who participated in 4 protocols using naked DNA VEGF gene transfer and were followed for up to 33 months after gene therapy.
However, the testing of this concept has not been limited to animal models. A total of 42 patients have now undergone direct intra-arterial gene transfer of naked DNA encoding VEGF to a freshly injured arterial surface. In 12 of these 42 patients, phVEGF165 was administered to normal or moderately diseased arterial segments by using a hydrogel-coated angioplasty balloon to promote therapeutic angiogenesis. Follow-up angiography and intravascular ultrasound showed no evidence of disease progression after gene transfer. In 30 other patients, the same delivery strategy was used to accelerate reendothelialization after percutaneous revascularization of femoral arteries occluded or severely narrowed by advanced atherosclerosis. Follow-up examination up to 48 months after gene transfer disclosed no evidence of new atherosclerotic lesion development, and the incidence of restenosis was at the very least no higher, and perhaps lower, than that observed among contemporary control subjects. Similar findings have been reported in a smaller series of patients undergoing coronary arterial gene transfer of adenovirus encoding for VEGF.

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**Vascular Malformations**

Experiments performed by Springer et al in immunocompromised mice disclosed the development of hemangioma after implantation of myoblasts transduced with a retroviral vector overexpressing VEGF into skeletal muscle. Similar findings were observed when the experiment was repeated in murine hearts. Although the impact of primary- versus later-passage cells used on the outcome of these experiments remains to be clarified, the authors speculated that the protracted, locally concentrated, high levels (200 pg/mL) of VEGF were responsible for hemangioma formation. Consistent with this interpretation, Schwarz et al observed similar structures when a bolus of 500 μg VEGF165 plasmid was

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Species</th>
<th>Target Vessel</th>
<th>Trauma</th>
<th>VEGF Gene</th>
<th>Protein</th>
<th>Mode of Delivery</th>
<th>Neointimal Thickening</th>
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<tr>
<td>1 Asahara</td>
<td>1995</td>
<td>Rat</td>
<td>Carotid artery</td>
<td>Balloon</td>
<td>+</td>
<td>Dwell</td>
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<tr>
<td>2 Asahara</td>
<td>1996</td>
<td>Rabbit</td>
<td>Femoral artery</td>
<td>Balloon</td>
<td>+</td>
<td>Balloon catheter</td>
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<td>+</td>
</tr>
<tr>
<td>3 Lazarous</td>
<td>1995</td>
<td>Dog</td>
<td>Femoral artery</td>
<td>Balloon</td>
<td>+</td>
<td>IV</td>
<td></td>
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<tr>
<td>4 Van Belle</td>
<td>1997</td>
<td>Rabbit</td>
<td>Iliac artery</td>
<td>Balloon/stent</td>
<td>+</td>
<td>Balloon catheter</td>
<td></td>
<td>+</td>
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<tr>
<td>5 Van Belle</td>
<td>1997</td>
<td>Rabbit</td>
<td>Iliac artery</td>
<td>Balloon/stent</td>
<td>+</td>
<td>Balloon catheter</td>
<td></td>
<td>+</td>
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<tr>
<td>6 Inoue</td>
<td>1998</td>
<td>Human</td>
<td>Coronary</td>
<td>None</td>
<td>+</td>
<td>Autopsy specimens</td>
<td>+*</td>
<td></td>
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<tr>
<td>7 Luo</td>
<td>1998</td>
<td>Rabbit</td>
<td>Vein graft</td>
<td>Surgery</td>
<td>+</td>
<td>Topical</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>8 Isner</td>
<td>1998</td>
<td>Human</td>
<td>SFA/profunda</td>
<td>Balloon</td>
<td>+</td>
<td>Balloon catheter</td>
<td></td>
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<tr>
<td>9 Vale</td>
<td>1998</td>
<td>Human</td>
<td>SFA</td>
<td>PTA</td>
<td>+</td>
<td>Balloon catheter</td>
<td>+†</td>
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<td>10 Laitinen</td>
<td>2000</td>
<td>Human</td>
<td>Coronary</td>
<td>PTCA</td>
<td>+</td>
<td>Balloon catheter</td>
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<td>11 Henry</td>
<td>1999</td>
<td>Human</td>
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<td>+</td>
<td>IC/IV</td>
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<td>12 Hiltunen</td>
<td>2000</td>
<td>Rabbit</td>
<td>Aorta</td>
<td>Balloon/Chol</td>
<td>+</td>
<td>Balloon catheter</td>
<td>+</td>
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<td>13 Celletti</td>
<td>2001</td>
<td>Mouse, rabbit</td>
<td>Aorta</td>
<td>Chol</td>
<td>+</td>
<td>IP/IM</td>
<td></td>
<td>+</td>
</tr>
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</table>

PTA indicates percutaneous transluminal angioplasty; SFA, superficial femoral artery; PTCA, percutaneous transluminal coronary angioplasty; Chol, cholesterol; IC, intracoronary; IV, intravenous; IP, intraperitoneal; and IM, intramuscular.

*Increased intimal thickening associated with increased VEGF immunostain.
†Versus historical controls.

**TABLE 2. Studies Regarding VEGF and Neointimal Thickening**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Species</th>
<th>Target Vessel</th>
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<td>1996</td>
<td>Rabbit</td>
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<td>11 Henry</td>
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<td>None</td>
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<td>IC/IV</td>
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<tr>
<td>12 Hiltunen</td>
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<td>Rabbit</td>
<td>Aorta</td>
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injected into a single site in a rat infarct model; however, when the experiment was repeated with 250 μg, no hemangiomas were observed.62

Preclinical and clinical studies of gene therapy performed to date suggest that levels and duration of gene expression that have been investigated for therapeutic angiogenesis transfer are unassociated with hemangioma formation. Ischemic limbs and hearts harvested at necropsy after gene transfer, for example, have disclosed no evidence of hemangioma formation in preclinical studies of nonviral,17,63,64 adenoviral,28,29,65 or even adenovirus-associated viral66 gene transfer. Likewise, clinical evidence of vascular malformations, including analysis of amputated limbs1 and explanted hearts,5 has thus far been limited to a single report17 of transient telangiectasia, which developed in the distal limb after arterial gene transfer of VEGF165. The common feature in these preclinical and clinical studies is a more physiological level and duration of gene expression.

Neoplasms

Given the seminal work of Folkman et al67 in identifying angiogenesis as a target for inhibiting the growth and/or survival of tumors, it is understandable that strategies of therapeutic angiogenesis would raise concerns regarding the potential for stimulating carcinogenesis. However, this concern ignores the fundamental dictum that “angiogenesis in necessary but insufficient” by itself to promote neoplastic growth. Indeed, there is little evidence from preclinical studies to support the notion that administration of angiogenic growth factors, per se, is sufficient to stimulate the growth of neoplasms. Unpublished experiments from the laboratory of Michael O’Reilly, MD (oral communication, December 2000) in fact have shown that intraperitoneal administration of high-dose VEGF or FGF-2 protein to mice with B-16/F-10 melanoma failed to affect tumor growth or spread.

In the case of human cardiovascular trials of gene transfer, attempts to localize delivery of the transgene coupled with the fact that many of the angiogenic gene products bind avidly to matrix glycoproteins have resulted in circulating levels of the angiogenic growth factor that either are not measurable or are in the picogram per milliliter range.1,2,4 Moreover, the duration of even the latter level of circulating gene product is typically limited to <4 weeks in trials performed to date.1,2,4 Although it is unlikely that such low levels of growth factor circulating for such a limited time would be sufficient to promote the growth of a dormant neoplasm that would exhibit sustained growth after extinction of gene expression, this remains a potential risk of gene therapy involving an endothelial cell mitogen.

Review of available published data regarding this potential risk is reassuring, albeit limited to short-term follow-up of small numbers of patients. In our own clinical experience with 88 subjects who have undergone VEGF gene transfer for critical limb ischemia, the cumulative 7-year incidence of cancer has been limited to two patients with bladder cancer and one with liver and brain metastases from an unknown primary. Among 85 patients undergoing VEGF gene transfer for myocardial ischemia, neoplasms have been diagnosed in two patients followed for up to 3 years. Among 79 patients undergoing intracoronary gene transfer of Ad/FGF-4,28 new neoplasms were identified in two treated patients (versus no placebo patients) 69 and 267 days after gene transfer.

Because this typically elderly group of patients may be at higher risk for cancer to begin with, it will take considerable follow-up of large cohorts to accurately judge the magnitude of this risk. The Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis (VIVA) trial69 of recombinant VEGF protein therapy for patients with myocardial ischemia provides a useful perspective in this regard. Among 178 patients randomized to low-dose VEGF, high-dose VEGF, or placebo, three patients developed new evidence of cancer (fatal in two of three) within the 180-day time period of the trial; by serendipity, all three patients had been randomized to the control arm. In the Therapeutic Angiogenesis With Recombinant Fibroblast Growth Factor-2 for Intermittent Claudication (TRAFFIC) trial,70 newly diagnosed neoplasms were limited to one patient in the placebo group.

The difficulties of assessing the risk of neoplastic growth are exemplified by the pathological and CT findings shown in Figure 5 from a patient at our institution participating in a trial of VEGF gene transfer to prevent restenosis.14 One day after gene transfer, an abdominal ultrasound examination that was performed to rule out an abdominal aortic aneurysm fortuitously identified a hypernephroma. The involved kidney was resected, and the patient remains alive and well without evidence of recurrent disease at the 3-year follow-up. Had the ultrasound examination not been performed, however, the relationship of the tumor to gene transfer would have been uncertain.

Retinopathy

Angiogenic growth factors, including FGF-2 and (more recently) VEGF, have been implicated in the development of proliferative diabetic retinopathy and macular degeneration.71–74 Transgenic mice engineered to overexpress VEGF within the photoreceptors developed retinal neovascularization extending into the subretinal space.75 Baffi et al76 observed choroidal neovascularization after focal injection of an E1-deleted adenoviral vector encoding VEGF165 into the subretinal space of Long-Evans rats. Subretinal injection of adenovirus encoding murine VEGF165 at a dose 10 000 times greater than that used by Baffi et al resulted in intense leakage on fluorescein angiography.77 However, the risk for compromised vision posed by the administration of genes encoding for VEGF or other proangiogenic gene products at remote extraocular sites is currently unknown. Accurate assessment of such risks is confounded both by the absence of data regarding the potential for circulating gene products (particularly proteins circulating for a brief duration at low levels) to cross the blood-ocular barrier and by the lack of animal models of diabetes-induced retinal neovascularization.

Clinical studies to date, including patients with diabetes and a previous history of retinopathy, have disclosed no evidence to support the notion that exacerbation of ocular pathology is a risk of angiogenic growth factor administration. In the VIVA trial, for example, only one patient developed reduced visual acuity during clinical follow-up, and this patient again had been fortuitously randomized to placebo treatment.69 Extensive ophthalmologic monitoring of patients enrolled in phase 1 trials of FGF-2 recombinant
protein for lower extremity and myocardial ischemia performed at NIH disclosed no evidence of ocular complications (K.G. Csaky, MD, PhD, oral communication, December 2000). In the TRAFFIC trial of recombinant FGF-2 protein, unspecified “retinal disorders” were limited to one patient in the placebo group and another in the single-dose group.70 Although no systematic study has been published to date, we are unaware of any cases in which patients with VEGF-producing tumors, in whom relatively high levels of circulating VEGF protein may be observed for considerable periods of time, have been reported to develop ocular pathology.

Specifically with regard to gene therapy, we prospectively studied 129 patients to determine whether the administration of naked plasmid DNA encoding VEGF would have adverse systemic effects involving eye-ground pathology (P. Vale, MD, unpublished data, April 2001). Patients were included in that study if they were enrolled in one of four gene transfer trials conducted at our institution (intramuscular gene transfer for critical limb ischemia \(n=51\), arterial gene transfer for restenosis \(n=30\), and operative \(n=42\) or catheter-based \(n=6\) gene transfer for myocardial ischemia). All patients underwent direct ophthalmoscopic examination and more detailed retinal evaluation with a 90-diopter lens through the dilated pupil. Diabetic subjects were also examined with fluorescein dye. Patients were studied at baseline, at 3 to 6 months after gene transfer, and then yearly by their ophthalmologist if they were diabetic. Patients also underwent funduscopic examination at each follow-up visit for the respective protocols.

Of the 129 patients (68% male, aged 23 to 84 years), diabetes was present in 44 (34%) of the subjects (mean age 66.3 ± 1.4 years). Of the 44 diabetic patients, all had adult-onset type 2 diabetes mellitus (15 [34%] were on insulin, and 29 [66%] were on oral therapy), with an average disease duration of 10 years (mean 9.7 ± 1.2 years, range 5 to 20 years). Eighteen diabetic patients had evidence of simple diabetic retinopathy that had been stable for >12 months. Four patients had associated subclinical unilateral cataracts. No patients had active neovascularization, retinal exudates, or hemorrhages at the time of gene transfer. Four patients had undergone laser therapy for early proliferative retinopathy >12 months before gene transfer. In none of the diabetic patients was there evidence of progression of retinopathic changes to proliferative disease; this was the case both in those with preexisting background retinopathy and in those with normal eye-ground examinations before gene transfer. Likewise, no evidence of neovascular pathology was observed among nondiabetic subjects. Visual acuity remained unchanged from baseline in all patients at the latest follow-up.

Finally, it must be underscored that with close monitoring, the development of diabetic retinopathy can typically be promptly and successfully treated with laser irradiation; however, age-related macular degeneration has thus far proved refractory to most currently available therapeutic interventions.

Edema

VEGF was discovered as a tumor-secreted factor that augments vascular permeability, accounting for its original designation as vascular permeability factor.78 Indeed, the permeability-enhancing effects of VEGF have been estimated to be 50 times greater than those of histamine.79

The possibility that clinical applications of VEGF might be complicated by enhanced vascular permeability has thus constituted a concern for gene transfer strategies of therapeutic angiogenesis in particular. This concern has been recently fueled by experiments performed in transgenic mice engi-
neered to overexpress VEGF±angiopoietin. Overexpression in the skin of VEGF alone yielded mice with evidence of leaky vessels, whereas coexpression ofangiopoietin-1 resulted in leakage-resistant vessels. In subsequent experiments, these authors reported that the permeability-enhancing effects of adenoviral gene transfer of VEGF were associated with lethal consequences (that could be ameliorated by coexpression of angiopoietin-1) and interpreted these findings to suggest that enhanced permeability constitutes a serious risk for patients undergoing VEGF gene transfer.

To investigate the magnitude of this risk, we prospectively evaluated 90 patients with peripheral artery disease undergoing gene transfer of naked plasmid DNA encoding VEGF for clinical evidence of enhanced vascular permeability. Edema was not observed in any of 28 patients treated for claudication. Instead, the development of edema was confined to patients with critical limb ischemia, 4 (24%) of 17 patients with rest pain and 27 of (60%) of 45 patients with gangrene. The fact that the patients in whom tissue ischemia was induced only with walking failed to develop edema but that edema was observed in nearly one half of the patients with resting ischemia suggests that the permeability-enhancing effects of VEGF are directly and/or indirectly potentiated by tissue ischemia.

Clinically apparent peripheral edema typically developed within 3 weeks after gene transfer and corresponded temporally to an increase in circulating levels of VEGF, consistent with the time course of gene expression (2 to 3 weeks) established for this plasmid in preclinical animal studies. Edema was promptly attenuated after the administration of oral diuretics and resolved completely within 2 to 4 weeks after the initiation of therapy. Similar findings have been seen in patients with critical limb ischemia receiving Ad/HIF-1α/VP-16.

In contrast to the murine experiments described above, evidence of life- or limb-threatening edema has not been described in any of these patients or in any patients receiving VEGF as recombinant protein or via gene transfer for myocardial ischemia. Comparison of this clinical experience with the animal experiments once again suggests that the differing outcomes may be attributable to the duration and magnitude of VEGF overexpression. In the case of the transgenic mice, VEGF overexpression was initiated during gestation and continued unabated throughout the lifetime of the animal. In the case of the adenoviral gene transfer, the treatment regimen was designed to produce circulating levels of VEGF that were up to 6 logs greater than those measured in patients undergoing either naked DNA or adenoviral gene transfer. Therefore, these findings support the notion that the safety of gene transfer in general, including VEGF in particular, is optimized by a brief duration and suitably low levels of localized gene expression.

The extent to which edema may complicate gene transfer of angiogenic growth factors other than VEGF remains to be clarified. The Miles assay, which has been used previously to document VEGF-enhanced permeability, shows no evidence of similarly enhanced permeability for other angiogenic growth factors, including FGF-1, FGF-2, scatter factor, granulocyte-macrophage colony–stimulating factor, transforming growth factor-β, placental growth factor, and platelet-derived growth factor-BB. Parenthetically, it is likely that the results described here for the 165-isoform of VEGF are generic to other isoforms and/or homologues of the VEGF-A gene; the VEGF C gene, for example, tests positive in the Miles assay, and early clinical trials of VEGF-C gene transfer for patients with critical limb ischemia have also resulted in clinically apparent lower extremity edema (I. Baumgartner, MD, unpublished data, March 2000).

**Additional Safety Concerns**

**Hypotension**

Several angiogenic growth factors, including VEGF, FGF-1, and FGF-2, are recognized to be potent stimulators of NO production, apparently mediated via growth factor–induced upregulated Akt phosphorylation of NO synthase. Indeed, data from several experimental studies suggest that NO constitutes an important if not critical downstream mediator of angiogenesis.

**Arrhythmias**

The possibility that neovascularization might promote local heterogeneity of ischemic myocardium, resulting in unstable reentry, slowed conduction, or altered repolarization constitutes a potential complication of gene transfer strategies intended to promote therapeutic angiogenesis. To date, there are neither preclinical nor clinical data indicating that life-threatening arrhythmias may develop after myocardial gene transfer of genes encoding angiogenic growth factors.

**Catheter Delivery**

Preliminary reports have disclosed no adverse consequences of arterial gene transfer. More recent applications of myocardial gene transfer have incorporated the concept of catheter-based gene transfer as a means of performing intra-muscular gene transfer for therapeutic angiogenesis and heart failure. Preliminary applications in patients undergoing VEGF gene transfer for therapeutic angiogenesis have disclosed no complications in 25 patients receiving 6 injections each (total 150 injections) followed for ≥1 year.

**Future Perspective**

Phase 1 studies are crucial for providing the initial evidence regarding the safety of a novel therapy. Indeed, only with such evidence can further testing proceed. Analysis of available data in phase 1 trials of cardiovascular gene therapy performed to date is encouraging in this regard. Mortality figures for these initial trials are in most cases equal to or superior to historical and contemporary controls. Likewise, evidence of serious morbidity is limited. The favorable outcomes with regard to mortality and morbidity are particularly reassuring, given the fact that these trials were in most
cases composed of high-risk patients with poor prognoses. Because the various vectors and transgenes are studied in larger numbers of patients in subsequent phase 2 trials, it is possible that the threshold for testing these therapies on less desperately ill patients may be progressively reduced (Figure 6). Inclusion of such a broader range of candidates will ultimately provide more information regarding the universe of patients that may be treated safely but also benefit from this novel therapeutic paradigm. Similarly, it is possible that with such data in hand, the pace of enrollment, until now quite restrictive compared with that in clinical trials of novel recombinant proteins or devices, may be permitted to accelerate. All of this assumes, of course, that the analyses presented in the present review are formally confirmed in reviews performed by the appropriate Federal agencies, including the NIH and Food and Drug Administration (FDA).

In this regard, governmental proposals have been recently crafted that aim to harmonize, in explicit fashion, reporting requirements for both agencies. The FDA proposal, if adopted, would have the effect of making public all adverse events reported to the FDA involving patients enrolled in gene transfer protocols. Although this policy contrasts with that governing public disclosure of adverse events involving drugs or devices that account for most of the FDA portfolio, it has been argued that such a policy is required to restore public confidence in the clinical investigation of gene therapy after the Gelsinger death. Whatever the outcome of current deliberations, an explicit understanding of what is expected in terms of reporting requirements is likely to be regarded as a positive step by most investigators anxious to resolve a lingering source of potential confusion and restore public trust.

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