Depression of oxygen and nutrients provides a serious threat for tissue viability. Nature’s response to the development of profound tissue ischemia includes the up-regulation of angiogenic growth factors and mobilization of circulating cellular elements that together enable development of an accessory vasculature. The involved paradigm, not surprisingly, recapitulates many aspects of embryonic circulatory development. Multiple angiogenic factors and inhibitors have been implicated in the formation and correct patterning of functional blood vessels. Although hampered by several ambiguous clinical trials, transfer of genes encoding for proangiogenic factors is still an important therapeutic option to induce vascular growth after critical ischemia. Recent studies provide further evidence that neovascularization is regulated by infiltrating circulation cells, including bone marrow–derived endothelial progenitor cells or inflammatory cells. The use of “cell therapy” for therapeutic vasculogenesis will be discussed in the second part of this review article.

Gene Therapy for Neovascularization
Candidate Genes: Experimental Evidence
Potential therapeutic genes for improvement of angiogenesis or arteriogenesis include growth factors, which predominantly act on endothelial cells to promote endothelial cell proliferation, migration, and tube-forming activity (Table 1). Concomitantly, most of the growth factors render endothelial cells less sensitive for apoptosis induction. Among the first growth factors identified to improve angiogenesis were members of the vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) families. Meanwhile, various isoforms of VEGF have been identified. VEGF-A and its splice variants and VEGF-B preferentially activate the VEGF receptors 1 and 2, whereas VEGF-C and VEGF-D stimulate the VEGF receptors 2 and 3, thereby contributing to lymphangiogenesis. The VEGF receptors 1 and 2 are both expressed on endothelial cells and hematopoietic stem cells. The expression of VEGF receptors on hematopoietic stem cells in particular appears to be of major importance for VEGF-dependent regulation of endothelial progenitor cells (see Part II). More recently, another member of the VEGF family, the placenta-derived growth factor (PIGF), gained increasing attention as a potent direct activator of VEGF receptor 1 and amplifier of VEGF receptor 2 signaling. PIGF increases angiogenesis but also promotes a proinflammatory response leading to acceleration of atherosclerotic lesion formation, probably via activating VEGF receptor 1–positive monocytye cells.

The members of the FGF family (approximately 23) are multifunctional proteins that bind to various spliced isoforms of the FGF receptors. Activation of the FGF receptors, which are expressed on endothelial cells, smooth muscle cells, and myoblasts, stimulates the proliferation of the respective cell types. In particular, FGF-1, FGF-2, and FGF-4 are highly angiogenic and may act synergistically with VEGF.

Hepatocyte growth factor is another potent multifunctional protein that exerts high proangiogenic activity. It activates its receptor c-met, which is expressed on a variety of cells, including endothelial cells, but also on hematopoietic stem cells. Angiopoietin-1, neurotrophin nerve growth factor, erythropoietin, and insulin-like growth factor (IGF) are additional candidates for therapeutic angiogenesis (Table 1).

Some of the growth factors may enhance tissue regeneration not solely via the proangiogenic activity but also via promotion of stem/progenitor cell mobilization. VEGF, angiopoietin-1, and erythropoietin mobilized endothelial progenitors from the bone marrow. Interestingly, hepatocyte growth factor in combination with IGF-1 was shown to mobilize tissue-resident cardiac stem cells, resulting in cardiac regeneration.

Several proangiogenic factors may act indirectly via the upregulation of VEGF and possibly other growth factors. This has been shown for the members of the hedgehog (Hh) protein family, which are morphogens regulating epithelial-mesenchymal signaling during several crucial processes of embryonic development. Particularly, sonic hedgehog (Shh) was shown to be a potent inducer of neovascularization after ischemia. Although the molecular mechanism has not yet been elucidated fully, first studies suggest that Shh acts via upregulating angiopoietins and VEGF.
### TABLE 1. Factors Augmenting Neovascularization: Potential Candidates for Therapeutic Angiogenesis, Arteriogenesis, and Vasculogenesis

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>Molecular Targets</th>
<th>Effects on Progenitor Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>VEGF receptors expressed on endothelial cells, monocytes, hematopoietic stem cells; stimulates proliferation, migration, and tube formation</td>
<td>Mobilization of EPC</td>
</tr>
<tr>
<td>PlGF</td>
<td>VEGF receptor 1 (cross talk with VEGF receptor 2)</td>
<td>Improves survival and differentiation of EPC</td>
</tr>
<tr>
<td>FGF</td>
<td>FGF receptors expressed on endothelial cells, smooth muscle cells, and myoblasts; stimulates proliferation</td>
<td>Mobilization of hematopoietic stem cells and EPC</td>
</tr>
<tr>
<td>Angiopoietin-1</td>
<td>Tie-2 receptor expressed on endothelial cells; enhances vessel maturation and stability</td>
<td>Included in EPC culturing media</td>
</tr>
<tr>
<td>HGF</td>
<td>c-met receptor expressed on various cells including endothelial cells, cardiac myocytes, progenitor cells</td>
<td>Mobilizes EPC and hematopoietic progenitor cells</td>
</tr>
<tr>
<td>IGF</td>
<td>IGF receptor expressed on vascular cells and satellite cells; enhances skeletal muscle regeneration</td>
<td>Attraction of tissue-resident cardiac stem cells</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Activates the Epo receptor, which is expressed on hematopoietic stem cells, EPC, endothelial cells, and cardiac myocytes; improves survival</td>
<td>Included in EPC culturing media</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Activates monocyctic cells, stimulates arteriogenesis</td>
<td>Mobilization of hematopoietic stem cells and EPC</td>
</tr>
</tbody>
</table>

**Chemokines**
- MCP-1: Promotes arteriogenesis by stimulating CCR-2 receptor on monocyctic cells.

**Transcription factors**
- HIF-1: Activation of gene expression (e.g., VEGF, VEGF receptor 2, erythropoietin, IG-2, and NO synthase)

**Extracellular matrix proteins**
- CCN family (e.g., Cyr61): Interaction with integrins
- Del-1: Integrin binding (αvβ5)
- HIG-2: Upregulation of HXO3

EPC indicates endothelial progenitor cells; HGF, hepatocyte growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MCP-1; monocyte chemoattractant protein 1; CCR-2, C-C chemokine receptor; and HIF-1, hypoxia-inducible factor 1.

A second class of candidate genes represents monocyte chemoattractant protein-1 and granulocyte-macrophage colony-stimulating factor, which both act on monocyctic cells to promote arteriogenesis.8 Another option is the use of transcription factors, which are known to promote angiogenesis by targeting various proangiogenic genes such as, for example, hypoxia-inducible factor-1α or early growth response protein-1 (EGR-1).9

Other proangiogenic factors activate integrin-dependent pathways to promote angiogenesis. These integrin-activatory proteins include the extracellular matrix protein Del-1, which coordinates integrin expression by homeobox genes.10 Additionally, the family of CCN comprises potent proangiogenic factors such as Cyr61, which binds to αvβ5, thereby promoting angiogenesis.11 Since integrin-matrix interaction is crucial for controlled vessel development, the temporal regulation of proteins activating or inhibiting integrin signaling is essential for the process of adult neovascularization.

Additionally, regulators of the wnt/frizzled pathway, as shown for the secreted frizzled-related protein FrzA, promote adult angiogenesis.12 This effect was shown to be independent of VEGF.

Finally, Akt/protein kinase B may also represent an attractive therapeutic option, not only for augmenting tissue perfusion but also via more protean effects. Akt expression was long ago shown to induce neovascularization in ischemic tissue13,14 but more recently appeared to restore or preserve tissue integrity in jeopardized myocardium, emphasizing the potential potency of exploiting signaling pathways.

**Therapeutic Vessel Growth for Critical Ischemia: Evidence From Human Trials**

The large unmet medical need of “no-option” patients—those with disabling ischemia despite optimal medical treatment, after all possibilities for conventional mechanical revascularization have been exhausted—propelled the development of biological revascularization. Initially, these attempts at a new mode of therapy were made with the use of angiogenic growth factors, either as recombinant protein or as gene therapy.

**Therapeutic Angiogenesis for Myocardial Ischemia**

Preliminary clinical trials established that the results obtained in human subjects with critical limb ischemia15–17 may extend to patients with myocardial ischemia (Table 2).18–20 In particular, investigations of therapeutic neovascularization in patients experiencing functional class III to IV angina refractory to medical therapy and not amenable to conventional revascularization have reported significant symptomatic benefit. Initial studies performed in our laboratory documented that symptomatic improvement in patients with myocardial ischemia was associated with improvement in the outcome of single-photon emission CT (SPECT)-Sestamibi myocardial perfusion imaging19,21; not only was there a reduction in the perfusion deficits associated with pharmacological stress, but...
large rest defects often resolved as well. These findings constituted objective evidence of improved myocardial perfusion after therapeutic neovascularization, including the possibility that foci of hibernating myocardium might be successfully rescued.

To determine whether the implications of SPECT imaging could be confirmed by an independent diagnostic technique, we used a novel strategy of catheter-based electromechanical assessment of myocardial perfusion (NOGA system, Biosense-Webster, J&J). This system utilizes electromagnetic field sensors to combine and integrate real-time information from percutaneous, intracardiac electrograms acquired at multiple endocardial locations. The resulting interrogations can be used to distinguish between infarcted and normal myocardium and permit online assessment of myocardial function and viability.

The collated electric and mechanical results of percutaneous electromechanical mapping (EMM) provide both an assessment of myocardial viability (ie, the presence of normal versus reduced voltage) and wall motion (presence of normal versus reduced fractional shortening). Validation of intracardiac signal recording and location accuracy has been established previously, both in vitro and in vivo. Clinical investigations have demonstrated that the mapping capabilities of the NOGA system may be used to distinguish between infarcted and normal myocardium. Gepstein et al and Ben-Haim et al found significantly lower linear local shortening (LLS) (L1.4%) and bipolar voltages (<2 mV) in infarcted versus noninfarcted myocardium. Furthermore, comparison with pathological specimens confirmed that the location and extent of infarction could be accurately defined by EMM.

These earlier findings were confirmed by Kornowski et al, who showed that patients with prior myocardial infarction had reduced unipolar (7.2±2.7 mV) and bipolar (1.4±0.7 mV) voltage recordings compared with patients without prior infarction (19.7±4.4 and 5.8±1.0 mV for unipolar and bipolar recordings, respectively) and that these patients had reduced local endocardial shortening compared with patients without prior infarction. Moreover, Kornowski et al demonstrated that mean voltage and LLS values are highest when measured in myocardial segments with normal perfusion and lowest when measured from segments with fixed perfusion defects; intermediate LLS (4% to 12%) and voltage (≥5 mV) recordings were documented for myocardial segments with reversible perfusion defects.

Resolution of rest defects observed in the SPECT scans after gene transfer is particularly intriguing. In this population

<table>
<thead>
<tr>
<th>Therapy and Reference</th>
<th>Route of Administration</th>
<th>Active Placebo, n/n</th>
<th>Patient Characteristics</th>
<th>Study Design</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF-1 protein18</td>
<td>Intramyocardial injection during CABG</td>
<td>20/20</td>
<td>Multi-vessel disease with distal LAD disease after LIMA</td>
<td>Phase I</td>
<td>12 wk</td>
</tr>
<tr>
<td>FGF-1 protein35</td>
<td>Intramyocardial injection via thoracotomy</td>
<td>20/0</td>
<td>Severe angina, NR</td>
<td>Phase I</td>
<td>6 and 12 wk</td>
</tr>
<tr>
<td>FGF-2 protein36</td>
<td>Peri-interventricular during CABG</td>
<td>8/0</td>
<td>NR, viable myocardium</td>
<td>Phase I</td>
<td>3 mo</td>
</tr>
<tr>
<td>FGF-2 protein37</td>
<td>Peri-interventricular during CABG</td>
<td>16/8</td>
<td>NR, viable myocardium</td>
<td>Phase I</td>
<td>3 mo</td>
</tr>
<tr>
<td>FGF-2 protein38,39</td>
<td>IV or intracoronary injection</td>
<td>59/0</td>
<td>CAD, NR, RD</td>
<td>Phase I</td>
<td>1, 2, and 6 mo</td>
</tr>
<tr>
<td>FGF-2 protein40</td>
<td>Intracoronary to left main</td>
<td>17/8</td>
<td>CAD and angina</td>
<td>Phase I</td>
<td>1 to 28 d</td>
</tr>
<tr>
<td>VEGF165 protein42,43</td>
<td>Intracoronary injection</td>
<td>251/86</td>
<td>CAD, NR, RD, EF &gt;30%</td>
<td>Phase I FIRST</td>
<td>90 d to 6 mo</td>
</tr>
<tr>
<td>VEGF165 protein44</td>
<td>IV injection</td>
<td>28/0</td>
<td>CAD, NR, RD</td>
<td>Phase I</td>
<td>60 d</td>
</tr>
<tr>
<td>VEGF165 protein45</td>
<td>Intracoronary + 3 IV injections</td>
<td>115/63</td>
<td>CAD, NR, RD</td>
<td>Phase II VIVA</td>
<td>60, 120 d, 1 y</td>
</tr>
<tr>
<td>Naked plasmid DNA/VEGF46</td>
<td>Intramyocardial via thoracotomy</td>
<td>30/0</td>
<td>CAD, NR, RSA</td>
<td>Phase I</td>
<td>2, 6 mo; 1 y</td>
</tr>
<tr>
<td>VEGF121/ADV47</td>
<td>Intramyocardial during CABG or via thoracotomy</td>
<td>21/0*</td>
<td>CAD, NR, RD</td>
<td>Phase I</td>
<td>30 d</td>
</tr>
<tr>
<td>VEGF121/ADV48</td>
<td>Intramyocardial via thoracotomy</td>
<td>10/0†</td>
<td>CAD, NR, RD</td>
<td>Phase I</td>
<td>30 d</td>
</tr>
<tr>
<td>VEGF-2 naked DNA47</td>
<td>Intramyocardial via thoracotomy</td>
<td>30/0</td>
<td>CAD, NR, RSA, CCS class 3/4</td>
<td>Phase I</td>
<td>30, 60, 90 d</td>
</tr>
<tr>
<td>VEGF-2 naked DNA48</td>
<td>Intramyocardial via EMMC in LV</td>
<td>6/3‡</td>
<td>CAD, NR, RSA, CCS class 3/4</td>
<td>Phase I</td>
<td>30, 60, 90 d</td>
</tr>
<tr>
<td>VEGF-2 naked DNA49</td>
<td>Intramyocardial via EMMC in LV</td>
<td>12/7</td>
<td>CAD, NR, RSA, CCS class 3/4</td>
<td>Phase VIII</td>
<td>30, 60, 90 d</td>
</tr>
<tr>
<td>FGF-4/adenoviral vector49</td>
<td>Intracoronary injection</td>
<td>60/19 (3:1)</td>
<td>RSA, &gt;1 open vessel, suitable for CABG/PTI</td>
<td>Phase I/II AGENT</td>
<td>4 and 12 wk</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; CCS, Canadian Cardiovascular Society; EF, ejection fraction; EMMC, electromechanical mapping catheter; FIRST, FGF-2 Initiating Revascularization Support Trial; IV, intravenous; LAD, left anterior descending artery; LIMA, left internal mammary artery; LV, left ventricle; NR, not candidate for revascularization; RD, reversible defect; RSA, refractory stable angina; PTI, percutaneous transluminal intervention; and VIVA, VEGF in Ischemia for Vascular Angiogenesis.

*Fifteen patients had CABG, and 6 had thoracotomy.
†Six patients had thoracotomy, and 4 had thoracoscopy.
‡Three control patients crossed over to active treatment.
of severely disabled, no-option patients, the rest defects were presumed to represent sites of myocardial scar associated with the clinical history of myocardial infarction in 13 of 13 patients. Partial or complete resolution of these rest defects after gene transfer is consistent with the notion that these preexisting defects constitute foci of hibernating myocardium and may have been successfully resuscitated as the result of therapeutic neovascularization.

The corresponding NOGA maps likewise showed reduced evidence of ischemia after gene transfer. EMM provides separate assessments of viability (endocardial voltage recording) and function (linear local shortening). Thus, those areas of the NOGA map that showed viable myocardium with impaired function before gene transfer versus viable myocardium with improved function after gene transfer support the notion that the defects that resolved on the SPECT scans constitute sites of hibernating myocardium that have been resuscitated as a result of myocardial neovascularization. These findings further confirm that left ventricular EMM may represent an independent diagnostic tool that may be useful for defining the myocardial consequences of improved perfusion.

**Percutaneous Gene Transfer for Therapeutic Angiogenesis in Patients With Myocardial Ischemia**

The aforementioned clinical findings, as well as preliminary studies performed in swine with myocardial ischemia, suggested that mapping the extent of ischemia may also be used online to direct percutaneous myocardial gene transfer. Such an adjunct may be particularly advantageous for optimizing low-efficiency strategies such as naked DNA gene transfer, in which EMM may direct injection of naked DNA to ischemic muscle, shown previously to yield higher levels of gene expression. We thus designed a pilot study to assess the feasibility, safety, and potential efficacy of catheter-based, percutaneous myocardial gene transfer of naked DNA encoding VEGF-2 administered via a novel needle-injection catheter and compared this in single-blind fashion with a mock procedure.

A steerable, deflectable 8F catheter incorporating a 27-gauge instrument was advanced percutaneously to the left ventricular myocardium of 6 patients with chronic myocardial ischemia. After safety and evidence of bioactivity were documented with this approach in this small pilot study, a second study, this time double-blinded, was performed in 19 patients before it was interrupted by the Food and Drug Administration. In this prospective, randomized pilot study there was a statistically significant greater improvement in Canadian Cardiovascular Society class in the active treatment group compared with control-treated patients; as of this date, nearly all patients have been followed for ≥1 year with no mortality and no morbidity related to the interventions. This has led to plans for a randomized trial of 400 patients to begin in 2004.

Additional evidence for the potential for angiogenic gene therapy is provided by the Angiogenic Gene Therapy (AGENT) study, in which adenovirus encoding FGF-4, administered by a straightforward intracoronary infusion, showed trends toward efficacy in an 84-patient pilot study. Large randomized trials of this therapy, applied in patients with class 2 and 3 angina, are under way.


**Key Words:** angiogenesis ■ angiogenesis ■ neovascularization
Therapeutic Angiogenesis and Vasculogenesis for Ischemic Disease: Part I: Angiogenic Cytokines
Douglas W. Losordo and Stefanie Dimmeler

doi: 10.1161/01.CIR.0000128595.79378.FA
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/21/2487

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/