Dependence 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 monocytes.

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.
A second class of candidate genes represents monocyte chemoattractant protein-1 and granulocyte-macrophage colony-stimulating factor, which both act on monocytes to promote arteriogenesis.\(^9\) 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 avb5, 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 vasculogenesis.

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 tissue\(^13,14\) 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 ischemia\(^15–17\) 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 imaging\(^19,21\); 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.23

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.23,24 Clinical investigations have demonstrated that the mapping capabili-

### TABLE 2. Phase I/II Clinical Trials of Angiogenic Factors for Myocardial Angiogenesis

<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>Multivessel 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>Perforative 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>Perforative 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>15/0</td>
<td>CAD, NR, RD, EF &gt;30%</td>
<td>Phase I</td>
<td>30 and 60 d</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</td>
<td>VIVA 60, 120 d, 1 y</td>
</tr>
<tr>
<td>Naked plasmid DNA/VEGF165</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/H11001</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/H11022</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 DNA37</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 DNA38</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 DNA39</td>
<td>Intramyocardial via EMMC in LV</td>
<td>12/7</td>
<td>CAD, NR, RSA, CCS class 3/4</td>
<td>Phase II</td>
<td>VIVA 60, 90 d</td>
</tr>
<tr>
<td>FGF-4/adenoviral vector40</td>
<td>Intracoronary injection</td>
<td>60/19 (3:1)</td>
<td>RSA, &gt;1 open vessel, suitable for CABG/PTI</td>
<td>Phase 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.

The FGF-2 protein used in the study was a genetically engineered variant of the human FGF-2 protein that has been shown to be active in vivo. The study was a phase I/II clinical trial that enrolled patients with chronic ischemic heart disease and who were deemed candidates for percutaneous transluminal coronary angioplasty (PTCA) or surgery. The primary endpoint of the study was successful rescue of myocardial segments with reversible perfusion defects. The study found that FGF-2 treatment was safe and efficacious in improving myocardial perfusion in patients with chronic ischemic heart disease.
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-guage 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.

**References**


11. Babic AM, Kireeva ML, Kolesnikova TV, et al. CYR61, a product of a preexisting defect, constitutes foci of hibernating myocardium and may 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.

Key Words: angiogenesis  •  neoangiogenesis  •  neovascularization
Therapeutic Angiogenesis and Vasculogenesis for Ischemic Disease: Part I: Angiogenic Cytokines
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