Molecular and Cell-Based Therapies for Protection, Rescue, and Repair of Ischemic Myocardium
Reasons for Cautious Optimism

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Despite the significant therapeutic advances of the past 25 years, coronary ischemic artery disease (CAD) remains the predominant cause of premature death.1,2 The prevalence of the disease imposes enormous financial strain on the healthcare system,2,3 calling for new approaches for treatment of CAD. The availability of cardiotropic vectors capable of long-term and stable protein expression4 and the recent isolation of progenitor cells with regenerative and angiogenic potential5,6 may provide opportunities for the design of novel therapies for protection and rescue of the myocardium from ischemia and failure. Cardio-protective gene therapy strategies have been effective in animal models of myocardial ischemia and reperfusion injury,7,8 and gene transfer of proangiogenic cytokines has been used as a strategy for rescuing ischemic myocardium.9 Transplantation of autologous progenitor cells is emerging as a potential option for the revascularization and repair of ischemic and infarcted myocardium.6 Notwithstanding these promising findings, there is pressing need for the development of safer and more effective vectors and the optimization and standardization of gene- and cell-based therapies.

In this article, we discuss the current preclinical and clinical advances in gene- and cell-based therapies for protection, rescue, and repair of the ischemic myocardium, with emphasis on strategies for protection of the myocardium from ischemia and reperfusion injury and for neovascularization and regeneration of ischemic and infarcted myocardium.

Gene Therapy for Protection of Myocardium at Risk

The development of gene therapies for acute myocardial infarction (MI) is not possible with the current vectors because the time required for transcription and translation of therapeutic genes exceeds the time window for successful intervention. For this reason, gene transfer of anticoagulant genes is not feasible as primary thrombolytic therapy for acute MI. Alternatively, a gene therapy strategy may be devised to provide long-term myocardial protection from ischemia-induced injury. This novel preventive gene therapy concept would protect the heart from future ischemia/reperfusion (I/R) injury, thereby minimizing the need for acute intervention in high-risk patients. Several cytoprotective genes may have potential as therapeutic targets to induce myocardial protection (Table 1).

Gene Therapy for Myocardial Protection From Oxidative Stress

Overexpression of antioxidant genes may be a useful strategy to potentiate endogenous antioxidant reserves and reduce oxidative stress–induced damage in the ischemic myocardium. We evaluated the feasibility of antioxidant enzyme gene transfer in long-term protection against I/R-induced oxidative injury using adeno-associated virus for intramyocardial delivery of heme oxygenase-1 (HO-1) gene in a rat model of myocardial I/R injury.7 HO-1 gene delivery to the left ventricular (LV) risk area several weeks before MI resulted in approximately 80% decrease in infarct size in association with decreases in oxidative stress, inflammation, and interstitial fibrosis and was accompanied by postinfarction recovery and normalization of ventricular dimensions,10 indicating that the gene therapy treatment prevents long-term negative remodeling of the infarcted myocardium. We observed similar results after gene transfer of the free-radical–scavenging enzyme extracellular superoxide dismutase.11 Efficient protection from I/R injury in animals has also been achieved by overexpression of other major antioxidant enzyme systems,12,13 as well as heat shock proteins14 and survival genes such as Bcl-215 and Akt16 (Table 1).

This preemptive gene therapy strategy for myocardial protection may also be useful in prevention of cardiac allograft rejection. Administration of HO-1 by adeno-associated virus to rat hearts before transplantation led to long-term allograft survival by preventing graft atherosclerosis, inflammation, and interstitial fibrosis.17 Others have demonstrated the therapeutic potential of gene transfer of immunomodulatory cytokines for local immunosuppression and induction of donor-specific tolerance.18
Gene Therapy for Myocardial Protection
From Inflammation
The inhibition of proinflammatory genes activated by I/R injury may offer an option for acute cardioprotection (Table 1). Pretreatment with a decoy oligonucleotide capable of inhibiting the trans-activating activity of nuclear factor-xB reduced myocardial infarct after coronary artery ligation in rats, whereas treatment with antisense oligonucleotide directed against intercellular adhesion molecule-1 was reported to prolong cardiac allograft tolerance and long-term survival when administered ex vivo before transplantation into the host. Thus, the use of oligonucleotides may be useful in treatment of acute myocardial ischemia and in cardiac transplantation as a tool for inhibiting pro-oxidant and proinflammatory genes activated by ischemia and reperfusion.

Gene Therapy for Protection of Vein Grafts
Protective gene therapy may also be useful in the engineering of atherosclerosis-resistant vein grafts to prevent graft failure after coronary artery bypass grafting (CABG). We have previously shown that treatment of jugular veins with antisense or decoy oligonucleotides against cell cycle–regulatory proteins cdc2 kinase and proliferating cell nuclear antigen or E2F-1, before carotid interpositional grafting inhibited graft atherosclerosis and improved endothelial function in atherosclerotic rabbits. More significantly, the gene therapy led to adaptive remodeling of the graft, yielding conduits that resembled normal arteries. These findings led to the PROject in Ex-Vivo veingraft ENGineering via Transfection (PREVENT)-1 study, a phase I, prospective, randomized, double-blind trial of human saphenous vein using ex vivo E2F decoy treatment before arterial interpositional grafting. The results of this trial, although preliminary, showed that E2F decoy treatment is safe and feasible for clinical application.

More recently, PREVENT-2, a randomized, double-blinded, placebo-controlled phase II trial, evaluated the effect of E2F decoy on CABG failure. The preliminary results confirmed the safety of E2F-1 treatment, and analysis of the secondary end points using quantitative coronary angiography and 3D ultrasound demonstrated improved patency and decreased neointimal size in the treated group up to 1 year after treatment. The ease with which the treatment can be incorporated during routine vein grafting procedures could potentially offer an efficient and cost-effective therapeutic strategy for prevention of postintervention vessel remodeling.

Gene Therapy for Rescue From Ischemic Heart Disease
Exogenous delivery of genes coding for proangiogenic growth factors may offer a potential alternative for the treatment of obstructive CAD in patients for whom percutaneous angioplasty or surgical revascularization has been excluded. (Table 1). Therapeutic angiogenesis has been demonstrated in myocardial ischemia by gene transfer of several angiogenic factors. Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor have been the factors most widely used in preclinical and clinical gene transfer protocols. Improve-

### TABLE 1. Targets for Gene-Based Therapy for Myocardial Protection and Rescue From Ischemia-Induced Injury

<table>
<thead>
<tr>
<th>Strategy/Therapeutic Target</th>
<th>Genetic Manipulation</th>
<th>Vector</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protection/prevention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant genes</td>
<td>HO-1, SOD, catalase, GPx</td>
<td>Overexpression</td>
<td>ADV, AAV, LV, α-virus</td>
<td>CAD, ACS, I/R injury 7, 10–13, 17</td>
</tr>
<tr>
<td>Heat shock proteins</td>
<td>HSP70, HSP90, HSP27</td>
<td>Overexpression</td>
<td>ADV, AAV, LV, α-virus</td>
<td>CAD, ACS, I/R injury 14</td>
</tr>
<tr>
<td>Survival genes</td>
<td>Bcl-2, Akt, HGF</td>
<td>Overexpression</td>
<td>ADV, AAV, LV, α-virus</td>
<td>CAD, ACS, I/R, HF 15, 16</td>
</tr>
<tr>
<td>Inflammatory cytokines, adhesion molecules, and TF</td>
<td>ICAM, VCAM, TNF-α, NF-κB</td>
<td>Inhibition</td>
<td>AS-ODN, Decoy ODN, ADV-AS-ODN, RV-AS-ODN</td>
<td>MI, I/R injury, graft atherosclerosis, transplantation 18</td>
</tr>
<tr>
<td>Proapoptotic genes</td>
<td>Bad, caspase inhibitorp53, Fas ligand</td>
<td>Inhibition</td>
<td>AS-ODN Decoy ODN ADV-AS-ODN</td>
<td>I/R injury, HF 8</td>
</tr>
<tr>
<td>Coronary vessel tone</td>
<td>eNOS, adenosine (P1, P3) receptors</td>
<td>Overexpression</td>
<td>RV, ADV, AAV (?)</td>
<td>CAD, I/R injury, HF</td>
</tr>
<tr>
<td><strong>Rescue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proangiogenic factors</td>
<td>VEGF121, VEGF165, FGF-1, FGF-2, FGF-4, FGF-5, HGF, eNOS Ang-1, MCP-1, G-CSF, PDGF-BB, IGF-1, IGF-2, HIF-1α/VP16, egr-1, Prox-1</td>
<td>Overexpression</td>
<td>Plasmid, ADV, AAV, LV (?)</td>
<td>CAD, MI, HF 9, 23—34</td>
</tr>
</tbody>
</table>

- TF indicates transcription factor; SOD, superoxide dismutase; GPx, glutathione peroxidase; HSP, heat shock protein; HGF, hepatocyte growth factor; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; TNF, tumor necrosis factor; NF, nuclear factor; eNOS, endothelial NO synthase; Ang-1, angiopeptin-1; MCP-1, monocyte chemotactic protein-1; G-CSF, granulocyte colony stimulating factor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; HIF, hypoxia inducible factor; egr-1, early growth response factor-1; ADV, adenovirus; AAV, adeno-associated virus; LV, lentivirus; AS-ODN, antisense oligonucleotide; RV, retrovirus; ACS, acute coronary syndromes; and HF, heart failure.
TABLE 2. Clinical Trials Using Gene Therapy for Therapeutic Angiogenesis in Myocardial and Peripheral Ischemia

<table>
<thead>
<tr>
<th>Trial Name/Authors</th>
<th>Trial Phase</th>
<th>Therapeutic Agent</th>
<th>Vector and Route of Administration</th>
<th>Therapeutic Target</th>
<th>Follow-Up</th>
<th>Therapeutic Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losordo et al. Circulation. 1998;38:2600</td>
<td>I</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Plasmid, intramyocardial</td>
<td>CAD not amenable to revascularization</td>
<td>10 weeks</td>
<td>↑ SPECT-sestamibi, ↑ Rentrop score, ↓ NTG use</td>
</tr>
<tr>
<td>Losordo et al. Circulation. 2003;105:2012</td>
<td>II</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Plasmid, transendocardial with NOGA catheter</td>
<td>CAD not amenable to revascularization</td>
<td>12 weeks</td>
<td>↑ CCS angina class, ↓ exercise duration, ↑ Seattle angina questionnaire</td>
</tr>
<tr>
<td>Vale et al. Circulation. 2001;103:2138</td>
<td>I</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Plasmid, transendocardial with NOGA catheter</td>
<td>CAD not amenable to revascularization</td>
<td>1 year</td>
<td>↑ SPECT-sestamibi, ↓ Rentrop score, ↓ NTG use, ↓ weekly angina attacks</td>
</tr>
<tr>
<td>Symes et al. Ann Thorac Surg. 1999;66:830</td>
<td>I</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Plasmid, Intramyocardial</td>
<td>CAD not amenable to revascularization with type III–IV angina</td>
<td>3 months</td>
<td>↑ SPECT-sestamibi, no rest ischemic pain, ↓ NTG use</td>
</tr>
<tr>
<td>Rosegart et al. Circulation. 1999;100:468</td>
<td>I</td>
<td>VEGF&lt;sub&gt;121&lt;/sub&gt;</td>
<td>Adenovirus, intramyocardial</td>
<td>CAD not amenable to revascularization</td>
<td>1 month</td>
<td>↑ SPECT-sestamibi, ↑ CCS angina class ▲ treadmill exercise</td>
</tr>
<tr>
<td>Hedman et al. Circulation. 2003;107:2635</td>
<td>I</td>
<td>VEGF&lt;sub&gt;121&lt;/sub&gt;</td>
<td>Adenovirus, intracoronary</td>
<td>CAD at time of PTCA</td>
<td>6 months</td>
<td>↓ coronary restenosis, ↑ myocardial KAT trial perfusion</td>
</tr>
<tr>
<td>Henry et al. VWA trial. Circulation.2003;107:1359</td>
<td>I</td>
<td>hVEGF&lt;sub&gt;165&lt;/sub&gt; protein</td>
<td>Intracoronary with intravenous supplement</td>
<td>CAD not amenable to revascularization</td>
<td>2 months</td>
<td>No change in ETT, ↓ angina episodes</td>
</tr>
<tr>
<td>Grimes et al. AGENT trial. Circulation.2002;105:1291</td>
<td>I</td>
<td>FGF-4</td>
<td>Adenovirus, intracoronary</td>
<td>Class II or III angina, &gt;1 vessel per patient</td>
<td>1–3 months</td>
<td>↑ ETT, improved stress ECG</td>
</tr>
<tr>
<td>Simons et al. FIRST trial. Circulation. 2002;105:788</td>
<td>I</td>
<td>FGF-2</td>
<td>Intracoronary bolus</td>
<td>Class II or III angina</td>
<td>90 and 180 days</td>
<td>↑ ETT, ↓ angina episodes at 90 days no differences at 180 days</td>
</tr>
<tr>
<td>Laham et al. J Am Coll Cardiol. 2000;35:2132</td>
<td>I</td>
<td>FGF-2</td>
<td>Intracoronary infusion</td>
<td>CAD not amenable to revascularization</td>
<td>1–6 months</td>
<td>↑ ETT, ↓ wall thickness and perfusion by MRI, improved quality of life</td>
</tr>
<tr>
<td>Unger et al. Am J Cardiol. 2000;85:1414</td>
<td>I</td>
<td>FGF-2</td>
<td>Intracoronary bolus</td>
<td>CAD with stable angina</td>
<td>1 month</td>
<td>↑ diameter of epicardial arteries</td>
</tr>
<tr>
<td>Kleiman et al. J Am Coll Cardiol. 2000;36:310</td>
<td>I</td>
<td>FGF-2</td>
<td>Intracoronary infusion</td>
<td>CAD not amenable to revascularization</td>
<td>6 months</td>
<td>No differences between placebo and treatment groups</td>
</tr>
<tr>
<td>Schumacher et al. Circulation. 1998;97:645</td>
<td>I</td>
<td>FGF-1</td>
<td>Intramyocardial</td>
<td>Three-vessel disease and distal LAD disease</td>
<td>12 weeks to 3 years</td>
<td>↑ anginogenicity distal to LAD, ↑ SPECT-sestamibi, ↓ NTG use</td>
</tr>
<tr>
<td>Baumgartner et al. Circulation. 1998;97:1114</td>
<td>I</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Plasmid, intramuscular</td>
<td>Critical limb ischemia</td>
<td>2–11 months</td>
<td>↑ ankle-brachial index, ↑ neovascularization, limb salvage</td>
</tr>
<tr>
<td>Makinen et al. Mol Ther. 2002;6:127</td>
<td>I</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Adenovirus, intraluminal after PTA</td>
<td>Critical limb ischemia and infrarenal occlusion</td>
<td>3 months</td>
<td>↑ neovascularization, ↑ ankle-brachial index</td>
</tr>
<tr>
<td>Isner et al. Lancet. 1996;348:370</td>
<td>I</td>
<td>VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
<td>Plasmid, intraluminal</td>
<td>Critical limb ischemia</td>
<td>3 months</td>
<td>↑ neovascularization and Doppler flow, ↑ ETT</td>
</tr>
<tr>
<td>Liederma et al. TRAFFIC trial. Lancet. 2002;359:2053</td>
<td>I</td>
<td>FGF-2</td>
<td>Intraluminal</td>
<td>Critical limb ischemia with intermittent claudiation</td>
<td>3 months</td>
<td>↑ ETT</td>
</tr>
</tbody>
</table>

GM-CSF indicates granulocyte-macrophage colony-stimulating factor; KAT, Kuopio angiogenesis trial; PTA, percutaneous transluminal angioplasty; LAD, left anterior descending coronary artery; NTG, nitroglycerin; CCS, Canadian Cardiovascular Society; and ETT, exercise tolerance time.

Regarding the efficacy and long-term sustainability of therapeutic effect. Intramyocardial delivery of a plasmid encoding VEGF<sub>165</sub> into the ischemic myocardium in 5 male patients 53 to 71 years of age with untreated CAD led to reduction in anginal symptoms and modest improvement in LV function, whereas adenovirus-mediated intramyocardial delivery of VEGF<sub>165</sub> into an area of reversible ischemia in the left ventricle as sole or adjunct therapy in patients undergoing conventional CABG led to improvements in regional ventricular function and wall motion in the region of vector administration in both groups of patients 1 month after gene transfer. All patients reported improvements in anginal symptoms, and 50% of the patients receiving VEGF<sub>165</sub> as the sole therapy reported increased treadmill exercise time. However, the sample size was too small to allow firm conclusions about the efficacy of the gene transfer strategy. More recently, Vale et al., using catheter-based delivery of VEGF-2 assisted by
electromechanical NOGA mapping of the left ventricle in patients with chronic myocardial ischemia, reported reductions in anginal attacks and improvements in myocardial perfusion for up to 1 year after gene delivery. This pilot study was followed by a phase 1/2 placebo-controlled, double-blinded trial using a dose-escalating design for catheter-mediated endocardial delivery of VEGF-2 in 19 patients with chronic untreatable myocardial ischemia. The authors reported significant improvements in Canadian Cardiovascular Society angina class and increase in treadmill exercise duration in the treated group compared with the placebo group.

In 2002, Grines et al reported the results of the Angiogenic GENE Therapy (AGENT) double-blinded, randomized, placebo-controlled trial using dose-escalating adenovirus-mediated intracoronary delivery of FGF-4 in 79 patients (mean age, 65 years) with CAD. Although the results showed a general trend toward increased treadmill exercise tolerance and improved stress echocardiograms at 4 and 12 weeks after gene transfer in the patients that received FGF-4 gene therapy, the trial was not sufficiently powered to detect statistically significant differences between the treated and placebo groups. More recently, the AGENT-2 trial assessed the effect of intracoronary adenovirus-mediated FGF-4 gene delivery on myocardial perfusion in 52 patients (mean age, 58 years) with stable angina at 8 weeks after gene transfer. Using stress-related reversible perfusion defect size as the primary end point, the authors reported improvement in perfusion in the treated group compared with the placebo group. However, the difference did not reach statistical significance, presumably because of the confounding effect of an outlier in the placebo group. Nevertheless, a greater number of patients in the treated group reported complete resolution of anginal symptoms.

Although the clinical findings have been inconclusive with regard to the efficacy of angiogenic gene therapy, we believe that larger and more adequately controlled trials are warranted. Stringent criteria must be applied in the selection of patients. Many of the candidates for therapeutic angiogenesis may have an impaired angiogenic response because of underlying endothelial dysfunction. It may be necessary to evaluate basal endothelial function in patients before enrollment in clinical trials, because nitric oxide plays an essential role in mediating the proangiogenic activities of several growth factors, including VEGF. In addition, objective end points for assessing efficacy need to be standardized and implemented, as well as measures to assess and overcome potential short- and long-term complications, such as hypertension, edema, retinopathy, and neovascularization of occult neoplasms. These problems could be circumvented by devising strategies to enhance the specificity of transgene expression. This may be achieved by incorporating tissue-specific and physiologically sensitive promoter sequences, such as hypoxia-responsive enhancer elements or engineered transcription factors capable of adjusting transgene expression to underlying changes in oxygen tension.

Cell-Based Therapy for Myocardial Ischemia

Endothelial progenitor cells (EPCs) offer another option for neovascularization of ischemic myocardium. The cells are thought to originate from a common hemangioblast precursor in bone marrow and under specific growth conditions may differentiate into mature endothelial cells and expand ex vivo for use in therapeutic applications. Two strategies have been used to achieve cell-based neovascularization in the myocardium. The most common approach involves injection of whole or culture-expanded cells isolated from the mononuclear cell fraction of bone marrow (BM-MNC) or peripheral blood (PB-MNC). The cells are then used without any additional manipulation, or they may be genetically modified with vectors expressing therapeutic genes and then delivered to the target area, where they may implant and promote new vessel growth. This approach has been applied to several animal models of myocardial ischemia and has been used in several small-scale clinical studies in patients with MI. (Table 3). For example, transplantation of autologous EPCs derived from CD34+ BM-MNC induced new vessel formation and improved LV perfusion and function in ischemic pig hearts. Others have reported that intravenous delivery of human CD34+ BM-MNC to nude rats with MI led to neovascularization of the infarcted myocardium, whereas implantation of bone marrow–derived Lin+ c-kit+ cells into the infarct border led to recuperation of infarcted myocardium and improvement in ventricular function in association with new vessel formation.

Another potential strategy for angiogenesis of ischemic myocardium in CAD involves the mobilization of EPC to ischemic regions using cytokines or conventional pharmacological agents used in treatment of CAD, such as statins. Orlic et al reported that the mobilization of bone marrow by granulocyte–colony stimulating factor or stem cell factor-1 led to a reduction in postinfarction mortality and functional recovery in mice with MI in association with regeneration and angiogenesis of the infarcted myocardium. Recently, statin therapy was shown to increase the number of EPCs in patients with stable CAD, suggesting that these drugs may exert their therapeutic effect, at least in part, by mobilizing EPCs to the ischemic myocardium.

Despite these encouraging findings, there are limitations to the therapeutic use of autologous EPC for treatment of tissue ischemia. The ability to expand these cells is limited by their scarcity in peripheral blood. Furthermore, functional impairment of EPCs has been reported in several pathologies, and an inverse correlation has been reported between the number of circulating EPCs and risk factors for CAD. This may limit the therapeutic usefulness of autologous EPC transplantation in these patients. Potential technical improvements to overcome these deficiencies may include isolation and expansion of EPCs from an alternate source, such as bone marrow, or the use of allogeneic EPCs from chord blood.

Cell-Based Therapy for Repair and Regeneration of Infarcted Myocardium

Because most adult cardiac myocytes are terminally differentiated, the regenerative capacity of the infarcted myocardium is limited. Myocyte loss attributable to infarction is initially compensated by hypertrophy of the surviving myocytes, which may help maintain structure and function. However, in time, these processes lead to maladaptive remod-
eling of the ventricle, which may progress to heart failure. Cell transplantation may offer a potential alternative for repair of infarcted myocardium. This strategy is based on the premise that repopulation of the necrotic myocardium with replication-competent cells will regenerate necrotic myocardium and rescue contractile function. Several cell sources have been used, including skeletal myoblasts, fetal and neonatal cardiomyocytes, embryonic stem cells, and bone marrow–derived adult stem cells. However, the therapeutic efficacy of cellular cardiomyoplasty has been inconsistent, and several key issues remain unresolved. For example, the optimal time for grafting after injury, the source and availability of cellular substrate, the delivery method, and the immune tolerance of the host to the grafted cells are important considerations.

The earliest attempt at cellular cardiomyoplasty for repair of injured myocardium was performed by Chiu et al, who reported that injection of autologous satellite cells into cryoinjured left ventricle in dogs led to formation of new myofibers with histological characteristics of cardiac fibers within the site of injury. However, it was recently reported that skeletal myoblasts grafted into left ventricle wall fail to transdifferentiate into cardiomyocytes or to form electromechanical coupling with native myocytes. Despite the poten-

### Table 3. Preclinical and Clinical Cell-Based Therapy for Therapeutic Angiogenesis of Ischemic Myocardium

<table>
<thead>
<tr>
<th>Target</th>
<th>Donor</th>
<th>Recipient</th>
<th>Type and Source of Cells</th>
<th>Method of Delivery</th>
<th>Therapeutic Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial ischemia</td>
<td>Swine</td>
<td>Autologous</td>
<td>CD31^+^, peripheral blood</td>
<td>Transendocardial</td>
<td>↑ Ren trop score, ↑ capillary density</td>
<td>39</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Swine</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Transendocardial</td>
<td>↑ capillary density, ↑ collateral flow, ↑ myocardial contractility</td>
<td></td>
</tr>
<tr>
<td>Hibernating myocardium</td>
<td>Swine</td>
<td>Autologous</td>
<td>MNC, peripheral blood</td>
<td>Transendocardial</td>
<td>↑ capillary density, ↑ collateral flow</td>
<td></td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Rat</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Intramyocardial</td>
<td>↑ capillary density</td>
<td>38, 39</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>Human</td>
<td>Nude rat</td>
<td>CD34^+^, peripheral blood</td>
<td>Intramyocardial</td>
<td>↑ capillary density, ↓ fibrosis, ↑ EF</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>Human</td>
<td>Nude rat</td>
<td>CD34^+^, bone marrow</td>
<td>Tail vein injection</td>
<td>↑ EF, ↑ capillary density, ↓ fibrosis, ↓ infarct size</td>
<td>40</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>GFP-mouse</td>
<td>Syngenic mouse</td>
<td>Lin c-kit^+^, bone marrow</td>
<td>Intramyocardial</td>
<td>↑ LVDP, ↑ capillary density, ↓ infarct</td>
<td>41</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>Rosa-mouse</td>
<td>Syngenic</td>
<td>SP cells, bone marrow</td>
<td>Systemic injection</td>
<td>No therapeutic effect</td>
<td>67</td>
</tr>
</tbody>
</table>

GFP indicates green fluorescent protein; MNC, mononuclear cells; SP, side population; CABG, coronary artery bypass grafting; and EF, ejection fraction.
tial problem of electrical isolation, there has been at least one report of autologous skeletal myoblast transplantation in patients with heart failure. Menasché et al reported that injection of autologous myoblasts derived from the vastus lateralis into the infarct scar during CABG in a 72-year-old patient with severe ischemic heart failure led to improved LV function 5 months after treatment. The same group has since treated an additional 4 patients and reported 13% improvement in ejection fraction and no occurrence of arrhythmia.

In an effort to avert some of the problems associated with skeletal myoblasts, some investigators have explored the use of fetal and neonatal myocytes. Cultured human fetal ventricular myocytes injected into the scar of immunosuppressed rats with MI formed stable grafts and survived up to 65 days after transplantation. Likewise, injection of neonatal cardiomyocytes from male Fisher rats into the infarcts of syngeneic female rats 1 week after left anterior descending ligation resulted in increased wall thickness in the region of the infarct and improved ejection fraction up to 6 months after cell grafting. In all cases, the transplanted cells were reported to have acquired biochemical and physical properties and physical properties of ventricular myocytes and form gap junctions with the adjacent cells.

The emergence of embryonic stem cell research has provided investigators with another potential source of cells for myocardial regeneration. Several groups have shown the ability of embryonic stem cell–derived cardiomyocytes to engraft and colonize damaged myocardium. However, there are serious ethical, moral, and legal limitations to the use of fetal, neonatal, or embryonic stem cell substrates for cardiac regenerative therapy. Access to these cells is highly restricted, and consistent and reproducible expansion of these cells is technically challenging. Furthermore, the cells are often rejected within days of transplantation, indicating the need for adjunctive immunosuppressive therapy.

The use of an adult self-renewing autologous source of progenitor cells with the potential for differentiating into cardiomyocytes would circumvent the problems associated with embryonic and fetal tissue. More significantly, the self-renewing capability of progenitor cells provides a readily available and sustainable substrate pool for autologous cell therapy protocols. Bone marrow–derived mesenchymal cells may be ideally suited for cellular cardiomyoplasty. These cells exhibit a high degree of plasticity and, under specific culture conditions, can differentiate into synchronously beating cardiomyocytes. Administration of BM-MNC has been reported to improve cardiac function in various models of myocardial injury. Tomita et al showed that transplantation of 5-azacytidine–treated bone marrow cells repopulated the scar and significantly improved LV function in cryoinjured rat hearts, whereas Toma et al reported that transplantation of human MSCs into the left ventricle of immunodeficient mice differentiated into cardiac myocytes without the need for myogenic differentiation before transplantation. Jackson et al reported that systemically administered side population cells into lethally irradiated mice can migrate to the myocardium and coronary vascular bed after MI and differentiate into cardiomyocytes and endothelial cells in the peri-infarct region. Orlic et al showed that delivery of bone marrow–derived c-kit/lin cells from green fluorescent protein–expressing male transgenic mice into syngeneic females with MI regenerated up to 68% of the infarct area, giving rise to myocytes and vascular structures expressing Y chromosome markers. More recently, several groups reported evidence of extracardiac progenitors in necropsy specimens of hearts obtained from subjects that had undergone sex-mismatched heart transplantation.

Evidence of resident progenitors in the heart has also been reported recently. Beltrami et al identified clusters of highly proliferating cells in the myocardium, consisting predominantly of undifferentiated lineage negative (Lin–) cells expressing stem cell markers c-kit (c-kit) and stem cell antigen 1 (Sca-1). These cells have clonogenic and self-renewing capabilities and are capable of differentiating into all myocardial cell types, including cardiomyocytes, endothelial cells, and vascular smooth muscle cells. The cells were reported to have regenerative potential, leading to significant myocardial regeneration on implantation of early passage cells into infarcted hearts from syngeneic animals, thus providing support that these cells are bona fide resident cardiomyogenic precursors. These findings suggest that the resident precursor cells may represent a mechanism for self-repair of the damaged myocardium. Such a mechanism could play a role in replacement of damaged or dying myocytes or in the renewal of myocytes lost as a result of biological turnover and cellular aging in the normal heart. However, the regenerative capacity of this putative self-repair mechanism has been questioned by several groups who have argued that the number of cardiac and extracardiac progenitors that migrate to the heart is insufficient to induce effective long-term regeneration of myocardium.

The mechanism of myocardial repair by local or transplanted cells has not been elucidated. The relative contribution of transdifferentiation and cell fusion to the regenerative process remains controversial. Using a Cre-Lox donor/recipient pair of transgenic mice, Oh et al reported equal contributions of differentiation and fusion to the regenerative process after intravenous administration of Sca-1 cells isolated from α-myosin heavy chain–Cre into Cre-dependent LacZ–expressing transgenic mice with MI. In contrast, Beltrami et al did not find evidence of cell fusion after transplantation of c-kit cardiac progenitors into infarcted rat heart.

Cell-Based Clinical Trials for Myocardial Repair and Regeneration

The clinical use of bone marrow cell transplantation in treatment of ischemic myocardium has recently been evaluated in several small-scale trials (Table III). Stamm et al reported that injection of autologous AC133+ BM-MNC into the infarct border during CABG in 6 patients with acute MI improved perfusion of the infarcted area and led to recovery of LV function 3 to 9 months after surgery. Strauer et al and Assmus et al reported that intracoronary delivery of unfractionated BM-MNC or PB-MNC 4 to 6 days after MI led to a reduction in infarct size and improvement in ventricular function and chamber geometry up to 4 months after transplantation. Two other groups reported recently that transcendocardial delivery of autologous BM-MNC using NOGA mapping led to significant

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improvements in LV perfusion and performance and reduced incidence of ischemic episodes in patients with end-stage ischemic heart disease\textsuperscript{45} or stable angina.\textsuperscript{46}

**Perspectives and Future Directions**

Several gene- and cell-based strategies with potential therapeutic value in the treatment of heart disease have evolved over the past decade. Although many of these experimental therapies have shown efficacy in animal models, only a select few cases have progressed to clinical trial to evaluate their safety and feasibility in treatment of CAD. However, these small-scale trials have not provided unequivocal evidence of their therapeutic efficacy. This may be attributed, at least in part, to the deficiencies of current vector and delivery tools.

There is pressing need for additional developments in vectors with improved safety and efficacy profiles. The future of myocardial gene transfer will require the use of nonimmunogenic tissue-specific vectors capable of expressing the therapeutic genes in a physiologically regulated fashion. New delivery devices and mapping techniques should improve the precision and specificity of gene delivery to the areas of interest and minimize systemic spillover. There is also need to standardize gene therapy protocols to allow proper evaluation and comparison of therapeutic benefits in different patient populations.

The outlook for cell-based regenerative therapies is promising. However, we caution against their premature use in the clinical setting until the issues of the timing of administration, the appropriate clinical condition (acute MI versus heart failure), the optimal cell number/composition, and, importantly, the safety of stem/progenitor cell protocols are better understood. Additional work will elucidate the mechanisms involved in mobilization, homing, integration, and survival of progenitor cells at the sites of implantation. This, in turn, will help define the optimal conditions for therapeutic application. We will also likely see greater reliance on cell-based gene therapy combination strategies. The question remains unsettled about the optimal therapeutic strategy for heart disease. Complex diseases such as CAD and MI may require a combination of cell transplantation and proangiogenic gene transfer for long-term viability of the rescued myocardium.

Finally, we believe that future advances in gene and cell therapies will benefit from developments in genomic research. Genomic profiling may be used to screen patients for disease-causing genetic polymorphisms and allow the design of tailor-made therapies for these patients. In addition, genomic research may help uncover the molecular mechanisms underlying stem cell mobilization, homing, and differentiation.

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