Refractory angina pectoris is a chronic disabling condition affecting ≈850,000 patients in the United States. It is characterized by frequent angina attacks unresponsive to maximal medical therapy and obstructive coronary artery disease not amenable to coronary revascularization. Although major progress has been made in medical therapy and cardiovascular interventions, up to 43% of patients continue to experience symptoms and 33% have positive exercise tests after angioplasty. It is now well recognized that these patients have concomitant microvascular disease, with reduced coronary and systemic flow reserve at a microvascular level and impaired endothelium-mediated vasorelaxation, that is, endothelial dysfunction. Currently, the treatment of these patients remains a major clinical challenge.

To address this large unmet therapeutic need, research has focused on biological strategies for refractory angina. A key effort is the use of cell therapy, which has the potential to restore the microcirculation and improve myocardial tissue perfusion by stimulating neoangiogenesis. In this regard, accumulating evidence supports the idea that cell-based therapy can improve clinical outcomes, including frequency of angina episodes, myocardial infarction (MI) rate, and exercise tolerance, in patients with refractory angina and, thus, should be subject to further trials to evaluate this treatment option for this patient population.

In this issue of Circulation Research, Khan et al present a comprehensive meta-analysis of cell-based therapy for refractory angina. Importantly, their analysis addresses the heterogeneity of the included trials, the problem of missing data, and limitations of the study. Single- and double-blinded, randomized clinical trials were included in this meta-analysis, comprising a patient population that had class III-IV Canadian Cardiovascular Society angina, were refractory to medical therapy, and were not coronary revascularization candidates (Table). The study included 192 patients who received cell therapy plus standard medical treatment and 161 patients who received only standard medical therapy. All 6 trials exhibited safety and efficacy. Three different cell types were examined: peripheral blood- or bone marrow-derived CD34+ cells in 3 trials, bone marrow-derived mononuclear cells (BM-MNCs) in 2 trials, and peripheral blood-derived CD133+ cells in 1 trial. Only PROGENITOR (Safety and Efficacy of Autologous Endothelial Progenitor Cell CD 133 for Therapeutic Angiogenesis) was a negative trial because it did not meet its primary end points of cardiovascular death, nonfatal MI, ischemic stroke, need for revascularization, and procedure-related complications. Five studies used the NOGA® cardiac navigation system for intramyocardial cell injections, and the remaining study used intracoronary delivery of CD34+ cells during cardiac catheterization.

The efficacy outcomes were frequency of angina episodes, Canadian Cardiovascular Society angina class, exercise tolerance, left ventricular function, change in antiangiinal medications, and quality of life. The effect of stem cell treatment on myocardial perfusion was assessed by single photon emission computed tomography. Clinical end points were combined into major adverse cardiac events and included MI, cardiac-related hospitalization, and mortality. Notably, the investigators found that cell-based therapy led to an improvement in myocardial perfusion (Figure). Pooled analysis from clinical trials demonstrated notable improvement in Canadian Cardiovascular Society angina class, left ventricular ejection fraction, use of antiangiinal medications, and a decreased risk of major adverse cardiac events. Finally, the occurrence of atrial and ventricular arrhythmias was also significantly decreased in the cell therapy group. Previous meta-analyses reported similar results of decreased angina frequency and MI rate and improved exercise tolerance. However, the meta-analysis by Khan et al advances the field by expanding the clinical parameters of the study and including results of myocardial perfusion as assessed by single photon emission computed tomography.

Cell-based therapy represents a potent biological drug that promotes tissue regeneration through mechanisms, including direct tissue transdifferentiation, cell–cell interaction with host tissue, and paracrine signaling. Because cell therapy promotes neoangiogenesis, therein lies the potential to restore the microvasculature and ameliorate refractory anginal symptoms. Mechanisms underlying promotion of neoangiogenesis with cell therapy involve release of paracrine factors, such as vascular endothelial growth factor, basic fibroblast growth factors, angiopoietins, and vascular endothelial growth factor family members. Other mechanisms include stimulation of endogenous angiogenesis by cell-secreted factors, which upregulate the expression of proangiogenic genes and promote the proliferation and migration of vascular cells.
factor, hepatocyte growth factor, angiopoietin-1, and others. Many of these angiogenic factors are also expressed by stem cells, and various studies have shown direct involvement of stem cells in neoangiogenesis in ischemic tissues, by increasing capillary density and creating anastomoses with the host circulation.13,14

BM-MNCs represent a heterogeneous population composed of hematopoietic stem cells, mesenchymal stem cells (MSCs), and endothelial progenitor cells. In preclinical studies, BM-MNCs augmented neoangiogenesis in a rabbit ischemic limb model by inducing collateral vessel formation and blood perfusion.15 Consistent with these findings, BM-MNCs injected into swine ischemic hearts produced a significant increase of regional blood flow and capillary density. The cardiac levels of basic fibroblast growth factor, vascular endothelial growth factor, and angiopoietin were significantly increased after stem cell injection compared with control.13 Moreover, angiogenic cytokines, like cardiac interleukin-1β and tumor necrosis factor-α, were significantly increased after BM-MNC administration and contributed to angiogenesis.14

Asahara et al first isolated endothelial progenitor cells from peripheral blood in 1997. Endothelial progenitor cells are a specific population of progenitor cells that home to sites of tissue injury and participate in angiogenesis by integrating with the host capillary vessels and forming capillaries.16 These cells are characterized by expression of surface markers, including FLK1 (VEGFR2), CD133, and CD34, and within a few weeks in tissue culture, they begin expressing endothelial

<table>
<thead>
<tr>
<th>Trial</th>
<th>Cell Type</th>
<th>Number of Patients, Total/Treated</th>
<th>Improved End Points</th>
<th>Unchanged End Points</th>
</tr>
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<tbody>
<tr>
<td>Jimenez-Quevedo et al 2014; Spain</td>
<td>Autologous peripheral blood–derived CD133+ cells</td>
<td>28/19</td>
<td>Myocardial perfusion&lt;br&gt;CCS angina class&lt;br&gt;Angina episodes/month&lt;br&gt;Nitroglycerin use/month</td>
<td>Cardiovascular death&lt;br&gt;Nonfatal MI&lt;br&gt;Ischemic stroke&lt;br&gt;Need for revascularization&lt;br&gt;Procedure-related complications</td>
</tr>
<tr>
<td>Losordo et al 2011; USA</td>
<td>Autologous peripheral blood–derived CD34+ cells</td>
<td>167/111</td>
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<td>Nitroglycerin use/day&lt;br&gt;CCS angina class&lt;br&gt;MACE</td>
</tr>
<tr>
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<td>Autologous bone marrow–derived CD34+ cells</td>
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<td>End systolic volume&lt;br&gt;End diastolic volume</td>
</tr>
<tr>
<td>Losordo et al 2007; USA</td>
<td>Autologous peripheral blood–derived CD34+ cells</td>
<td>24/18</td>
<td>Arrhythmia monitoring—no risk&lt;br&gt;Angina frequency*&lt;br&gt;Nitroglycerine use*&lt;br&gt;Exercise tolerance*&lt;br&gt;CCS angina class*&lt;br&gt;Myocardial perfusion*&lt;br&gt;Quality-of-life testing*</td>
<td>End systolic volume&lt;br&gt;End diastolic volume</td>
</tr>
<tr>
<td>Tse et al 2007; Hong Kong, Australia</td>
<td>Autologous BM-MNCs</td>
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<td>LV end-systolic volume&lt;br&gt;LV end-diastolic volume&lt;br&gt;CCS angina class</td>
</tr>
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BM-MNCs indicates bone marrow–derived mononuclear cells; CCS, Canadian Cardiovascular Society; LV, left ventricular; MACE, major adverse cardiac events; MI, myocardial infarction; and NYHA, New York Heart Association.

*Probability values were not shown because no power calculations to determine sample size were done.
markers, such as von Willebrand Factor and VE-cadherin. Their implantation has resulted in favorable effects on myocardial capillary density, perfusion, collagen deposition, and contractile function in a rodent MI model. Even in the setting of compromised macrovascular supply, improvement in microvascular and collateral perfusion can augment contractile function. A key mechanism for cell therapy is the stimulation of endogenous precursor cells, and in this regard, our group has shown that allogeneic MSCs injected into cardiac tissue stimulate endothelial progenitor cell bioactivity and restore endothelial function in patients with idiopathic dilated and ischemic cardiomyopathy. Accordingly, cell therapy may directly activate neoangiogenic pathways by direct activation of endogenous precursor cells.

Other Therapeutic Stem Cells for Patients With Refractory Angina

What is the best cell-based treatment for the patients with refractory angina? Despite the positive results of the meta-analysis by Khan et al, previous clinical trials with BM-MNC treatment failed to show efficacy in patients with acute MI and chronic ischemic cardiomyopathy, therefore, other cell types should be investigated in future clinical trials. Numerous clinical and preclinical studies demonstrated the therapeutic efficacy of MSCs. For instance, in the TAC-HFT (The Transendocardial Autologous Cells [hMSC or hBMC] in Ischemic Heart Failure Trial), MSCs improved cardiac function and structure in patients with chronic ischemic cardiomyopathy as compared with BM-MNCs and placebo. There is accumulating data from previous studies supporting a multifactorial mechanism of action by MSCs. These cells, when applied into a region of myocardial ischemia, can differentiate into smooth muscle cells and endothelial cells, leading to increased vessel density and improved cardiac function. Although preclinical data support substantial trilineage transdifferentiation (cardiomyocytes, vascular smooth muscle cells, endothelial cells) of MSCs in the porcine infarcted heart, the angiogenic effects of MSCs are enhanced by their paracrine actions involving secretion of vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived growth factor that influence adjacent cells and result in improvement of left ventricle remodeling, neovascularization, and tissue repair and decreased cell apoptosis, mitochondrial dysfunction, and microvascular dysfunction. The results of the PROMETHEUS trial (Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery) showed that MSCs injected into scarred myocardial segments that were not surgically revascularized produce significant improvement in myocardial perfusion, contractility, and reduction in scar size at 18 months after treatment. Additionally, MSCs can be important regulators of neovascularization by acting as pericytes, cells that stabilize the newly formed vasculature. Moreover, the paracrine mediators may also elicit autocrine effects on the biology of stem cells themselves. Therefore, the paracrine/autocrine mechanism extends the concept of the stem cell niche and includes the factors released by stem cells into the microenvironment controlling stem cell biology and tissue regeneration.

Cardiac stem cells are a cell population that reside in the heart and are characterized by the expression of c-kit (CD117), stem cell antigen 1, and Islet-1. In response to cardiac injury, cardiac stem cells promote increased vessel density, a mechanism associated with improved cardiac function. Interestingly, transplanted bone marrow-derived MSCs establish cell–cell interactions with host myocardium and stimulate endogenous c-kit+ cardiac stem cell differentiation and cardiomyocyte cell cycling. In preclinical studies, interactions between MSCs and cardiac stem cells enhance cardiac performance to a greater extent than MSCs alone and are associated with increased cardiac perfusion assessed by cardiac magnetic resonance imaging. These findings support the novel hypothesis that cell interactions activate stem cell niches and modulate the microenvironment toward regeneration.

Induced pluripotent stem cells are a novel cell type derived by reprogramming somatic cells via expression of exogenous transcription factors. They can differentiate into mature cell types, including vascular endothelial cells, which can be used for treatment of myocardial and limb ischemia, increasing capillary density through activation of paracrine mechanisms. Thus, this cell population is a potential candidate to treat refractory angina.

Allogeneic Cell Therapy

To date, clinical studies have used autologous cells to treat patients with refractory angina. There is intriguing evidence that...
allogeneic cell-based therapy produces similar safety and potentially greater efficacy in patients with ischemic and nonischemic heart disease. Indeed, POSEIDON (The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis Pilot Study) compared transendocardial injection of autologous versus allogeneic bone marrow-derived MSCs and reported similar safety profiles in the 2 groups and a significant reduction in left ventricular end diastolic volume in the allogeneic MSC treatment. Moreover, patients from the POSEIDON-DCM (Percutaneous Stem Cell Injection Delivery Effects On Neomyogenesis in Dilated Cardiomyopathy; NCT01392625) and TRIDENT (The Transendocardial Stem Cell Injection Delivery Effects on Neomyogenesis Study; NCT02013674) trials showed improved endothelial function 3 months after transendocardial administration of allogeneic, but not autologous, MSCs. The advantages of allogeneic cell products also include the relative ease of accessibility from young healthy donors, ability to expand in high volumes, and availability for infusion. Therefore, allogeneic cell therapy may be a superior alternative that can be used as an off-the-shelf product for patients with cardiovascular and other diseases.

**Conclusion and Future Perspectives**

Future studies should be designed to define the optimal cell type(s) to treat refractory angina, including combination cell therapy. One of the challenges in clinical trials has been the selection of appropriate time points, cell dosage, and route of administration. In addition, most cell therapy studies have not examined the most important clinical endpoints, that is, recurrent MI, cardiac-related hospitalizations, and mortality, and have only followed-up the patients for 6 or 12 months. Longer follow-up would allow for a better understanding of the long-term effects of cell therapy and mechanisms of neoangiogenesis activated by stem cells.

In summary, the meta-analysis by Khan et al showed promise for cell-based therapy for patients with refractory angina who are not candidates for revascularization. Although cell therapy is not yet a cure, it may provide benefits in terms of quality of life and longevity. The time for Phase 3 clinical studies has arrived to determine the most effective stem cell treatment for patients with refractory angina and also to understand the mechanisms by which the cells exert their therapeutic effects. Well-designed human studies with meaningful end points will help supplement the unanswered questions and provide support to keep this promising innovative field of research moving forward.

**Disclosures**

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Victoria Florea, Wayne Balkan, Ivonne Hernandez Schulman and Joshua M. Hare

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