

angina,<sup>1</sup> with 100 000 to 200 000 new cases per year.<sup>2</sup> The development of new therapies for this patient population therefore represents a suitable therapeutic target.

Laboratory and preclinical studies have provided evidence of the safety and potential efficacy of a strategy of intramyocardial transplantation of autologous CD34<sup>+</sup> stem cells for neovascularization of chronically ischemic myocardium.<sup>3-6</sup> Accordingly, we performed a pilot, first-in-human study to evaluate the safety and bioactivity of this approach in patients with coronary artery disease and intractable angina.

## Methods

### Study Design

This was a phase I/IIa, double-blind, placebo-controlled, randomized clinical trial. Patients were enrolled into 1 of 3 dose cohorts ( $5 \times 10^4$ ,  $1 \times 10^5$ , and  $5 \times 10^5$  CD34<sup>+</sup> cells/kg) versus placebo. All patients in all treatment groups, including placebo, received granulocyte colony-stimulating factor (G-CSF) at a dose of  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 5 days. We chose a low dose in the present study on the basis of the published dose-response relationship of the drug, which showed good mobilization at the  $5\text{-}\mu\text{g}/\text{kg}$  dose, and out of a desire to approach these patients with extensive coronary artery disease as conservatively as possible given prior data indicating that higher doses could be associated with an increased possibility of significant side effects.<sup>7</sup> Leukoapheresis was performed on the fifth day (Amicus, Baxter Healthcare, Deerfield, Ill) for collection of mononuclear cells. The cells were stored overnight at 4°C, and the following morning, the CD34<sup>+</sup> fraction was purified on a commercially available device (Isolex 300i, Baxter Healthcare) according to the manufacturer's instructions. Cells were then subjected to testing and were required to meet lot-release criteria that included sterility, viability, absence of endotoxin, and CD34 cell content. Once cells passed lot-release criteria, the patients underwent NOGA electromechanical mapping and intramyocardial injection of CD34<sup>+</sup> cells (suspended in saline plus 5% autologous serum; serum was added to support cell viability) versus cell diluent (saline plus 5% autologous serum) using the NOGA Myostar catheter (Biosense Webster, Diamond Bar, Calif).<sup>8</sup> Randomization codes were established by the study statistician and were revealed only to the stem cell laboratory technician responsible for separating the cells into aliquots or preparing the placebo material. The dose was divided into 10 injections of 0.2 mL per injection. Patients were discharged from the hospital the day after the injection procedure. Follow-up occurred at 1, 2, and 4 weeks and at 2, 3, 6, 9, and 12 months. Crossover was permitted if the patient met study entry criteria after 6 months of follow-up. The present report is restricted to the results of the initial randomized study population.

### Patient Population

Patients included for enrollment were required to be >21 years old with functional Canadian Cardiovascular Society (CCS) class III or IV angina; to have attempted "best" medical therapy, including long-acting nitrates, maximal use of  $\beta$ -adrenergic blocking agents, and calcium channel agents, without control of symptoms; and to be taking at least 2 antianginal medications. Patients were required to be considered noncandidates for conventional revascularization by the referring cardiologist, and an independent interventional cardiologist and cardiac surgeon reviewed the most recent (within 6 months) angiogram to verify ineligibility for revascularization. Patients were also required to have ischemia on nuclear perfusion imaging, to complete at least 1 minute but no more than 6 minutes of a standard Bruce protocol, and to experience angina/angina equivalent during the baseline exercise test. Key exclusion criteria included myocardial infarction within 30 days of treatment; successful coronary revascularization within 3 months of enrollment; documented transient ischemic attack within 60 days of treatment; severe aortic stenosis (aortic valve area  $<1.0 \text{ cm}^2$ ) or insufficiency ( $>2+$ ); severe mitral stenosis or severe mitral insufficiency; predominant congestive heart

failure symptoms; severe comorbidities associated with a reduction in life expectancy to less than 1 year; uncontrolled hypertension; joint disease, peripheral vascular disease, or chronic obstructive pulmonary disease that would limit walking on the treadmill; and patients with clinical evidence of a neoplasm within the last 5 years (other than nonmelanoma skin cancer or in situ cervical carcinoma).

## End Points

### Safety

An independent data safety monitoring board was assembled to review safety data in a timely manner. In addition to routine physical examination and laboratory testing, patients were also monitored with ECG and transthoracic echocardiography at routine intervals. Echocardiography was performed immediately after the injection procedure and before hospital discharge in all subjects.

### Arrhythmia Monitoring

All patients were required to have an implanted cardioverter-defibrillator already in place or to wear a temporary device (LifeVest) for 1 week before and 4 weeks after the injection procedure. Patients also underwent 24-hour Holter monitoring before and 1 week and 3, 6, and 12 months after injection. Patients with LifeVests did not have 1-week Holter monitoring, and those with implantable cardioverter-defibrillators did not undergo Holter monitoring.

### Efficacy

Bioactivity was assessed according to the following parameters: angina frequency, nitroglycerine (NTG) use, exercise tolerance (standard Bruce protocol), CCS class, single-photon emission computed tomography (SPECT) imaging (assessed at a core laboratory by J.U.), and quality-of-life testing.

### Statistical Analysis

Because this was a first-in-human study, no prior data were available on which to base power calculations. Accordingly, results are presented as change from baseline in patients assigned to placebo versus those assigned to cell injection. Because power calculations to determine sample size were not done, we do not show probability values, which would imply that a goal of this study was to document efficacy in this study. As a phase I/IIa study, a statistical assessment of efficacy is not the goal, and even if certain parameters revealed "significant" improvement, we believed that it would not be correct to display them, because these were not the prespecified aims of the study. Because crossover of placebo-assigned patients was permitted after 6 months, the analysis of efficacy parameters is restricted to 6 months after initial treatment assignment.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

### Patients

Twenty-four patients, including 5 women and 19 men with a mean age of 62.4 (range 48 to 84) years, were enrolled at 3 centers in the United States from December 2003 through March 2005. Because no dose-response effect was observed, data are presented as CD34<sup>+</sup> stem cell–treated patients versus controls (Table 1).

### Safety Analysis

Thirteen patients (54.2%) reported that their angina was transiently increased in frequency after the administration of G-CSF. Patients were instructed that an increase in angina might occur after administration of G-CSF as a result of increasing blood viscosity, metabolic demand, or increased platelet counts. The patient-reported increase in frequency and severity

**TABLE 1. Baseline Characteristics by Treatment Group**

	Placebo	CD34 <sup>+</sup> Cells/kg		
		5×10 <sup>4</sup>	1×10 <sup>5</sup>	5×10 <sup>5</sup>
Hyperlipidemia	4 (66)	6 (100)	5 (83)	6 (100)
Hypertension	3 (50)	5 (83)	5 (83)	4 (66)
Smoking history	5 (83)	6 (100)	3 (50)	4 (66)
Congestive heart failure	2 (33)	3 (50)	1 (17)	2 (33)
Myocardial infarction	3 (50)	3 (50)	4 (66)	4 (66)
Peripheral vascular disease	2 (33)	3 (50)	1 (17)	3 (50)
Diabetes mellitus	3 (50)	1 (17)	2 (33)	4 (66)
ACE inhibitor	3 (50)	3 (50)	5 (83)	4 (67)
β-Blocker	6 (100)	6 (100)	5 (83)	6 (100)
Statin	5 (83)	6 (100)	6 (100)	4 (67)
Prior PCI procedure(s)	6 (100)	4 (66)	6 (100)	5 (83)
Prior CABG surgery	4 (66)	6 (100)	6 (100)	5 (83)
Prior AICD implantation	2 (33)	1 (17)	2 (33)	0 (0)
Prior TMR or EECF	0 (0)	0 (0)	1 (17)	5 (83)

Values are expressed as n (%). ACE indicates angiotensin-converting enzyme; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; AICD, automated implantable cardioverter-defibrillator; TMR, transmyocardial revascularization; and EECF, enhanced external counterpulsation.

of angina occurred as early 1 day after the initiation of GCSF and persisted in some individuals until the day of apheresis. The only pattern that emerged was the return to baseline symptom frequency after apheresis. The increase in angina was manageable in all patients with the use of sublingual NTG. There were no cardiac enzyme elevations, myocardial infarctions, acute coronary syndromes, or deaths, some of which had occurred after administration of higher doses of GCSF in similar patient populations.<sup>9</sup> Because no GCSF control was available, we are unable to determine whether the reported change in symptom pattern was specifically drug related.

Serious adverse events (Table 2) were distributed among the treatment groups. One patient in the placebo group developed ventricular tachycardia during the mapping procedure. This individual had a history of sudden cardiac death and had a

**TABLE 2. Incidence of Serious Adverse Events**

	Placebo	CD34 <sup>+</sup> Cells/kg		
		5×10 <sup>4</sup>	1×10 <sup>5</sup>	5×10 <sup>5</sup>
Atrial arrhythmia	2 (33)	1 (17)	1 (17)	0
Ventricular arrhythmia	1 (17)	0	0	0
Angina exacerbation	3 (50)	2 (33)	3 (50)	2 (33)
Congestive heart failure	0	0	3 (50)	0
Respiratory arrest	0	2 (33)	0	0
Cerebrovascular accident	0	0	0	1 (17)
Peripheral vascular claudication	0	1 (17)	1 (17)	0
Bleeding/anemia	0	2 (33)	1 (17)	0
Renal insufficiency	2 (33)	0	2 (33)	0
Gastrointestinal complication	1 (17)	0	1 (17)	0
Endocrine/electrolyte disorder	1 (17)	0	2 (33)	1 (17)

Values are n (%).

previously implanted cardioverter-defibrillator. The patient was cardioverted successfully, and the remainder of the mapping procedure and injections were performed without incident. No further sustained ventricular arrhythmia occurred in this patient, and no arrhythmias were detected by implantable cardioverter-defibrillator, LifeVest, or Holter monitoring in any patient during or after the injection procedure.

**Efficacy Analysis**

**Angina Frequency**

At baseline, patients in the placebo group were experiencing 20.5±11.5 episodes of angina compared with 21.2±16.1 episodes of angina per week in the treatment group. At 3 months after injection, the frequency of angina was increased in the placebo group and decreased in the active treatment group (3 months: 27.0±23.8 episodes in the placebo group versus 9.6±13.3 in the treated group). At 6 months after injection, the frequency of angina was reduced in both placebo and CD34<sup>+</sup> cell-treated patients (6 months: 16.0±19.3 versus 8.6±10.3 episodes). At both time points, the CD34<sup>+</sup> stem cell-treated patients experienced a greater magnitude of reduction of symptoms (change from baseline: control, 3 months 6.5±15.2 and 6 months -4.5±20.1; CD34<sup>+</sup> cell treatment, 3 months -11.6±19.5 and 6 months -12.6±18.2; Table 3; Figure 1A).

**NTG Use**

At 3 and 6 months after injection, NTG use in the placebo group was increased compared with baseline (+8.8±20.7 and +4.8±37.9), whereas the CD34<sup>+</sup> stem cell-treated patients used less NTG at both time points (-9.8±10.8 and -8.1±14.7; Figure 1B; Table 3). The increase in NTG use in the control population, juxtaposed against a decrease in angina, may be a reflection of better utilization of NTG as a result of patient education in the course of trial participation.

**Exercise Tolerance**

At 3 months after injection, exercise time on the standard Bruce protocol was improved in placebo and active treatment groups compared with baseline (+0.3±2.1 and +0.5±1.3 minutes, respectively; Figure 1C). The CD34<sup>+</sup> stem cell-treated patients experienced a slightly greater magnitude of improvement in exercise time.

**CCS Class**

At 3 and 6 months after injection, the mean CCS class was reduced in the placebo and active treatment groups (3 months: -0.05±1.2 placebo versus -1.1±0.8 treated; 6 months: -0.8±1.7 placebo versus -1.4±1.0 treated; Figure 1D; Table 3). The CD34<sup>+</sup> stem cell-treated patients experienced a greater magnitude of reduction of CCS class at both time points. In addition, the percentage of patients experiencing at least a 2-CCS class decrease was greater in the CD34<sup>+</sup> cell-treated patients than in controls (16.7% decrease in placebo group versus 27.8% in treated group at 3 months and 33.3% decrease in placebo group versus 50% in treated group at 6 months).

**SPECT Perfusion Imaging**

SPECT imaging at 3 and 6 months after injection yielded inconsistent findings. For example, the automated summed

TABLE 3. Change in Angina, NTG, and CCS at 3 and 6 Months After Injection

Treatment Group and Maximum CD34 <sup>+</sup> Count/ $\mu$ L*	Maximum WBC Count $\times 10^3/\text{mm}^3$	Angina Change From Baseline		NTG Change From Baseline		CCS Change From Baseline		ETT Change From Baseline at 3 Months
		3 Months	6 Months	3 Months	6 Months	3 Months	6 Months	
<b>Placebo</b>								
11	11.4	0	25	0	25	0	1	-0.3
10	25.5	-15	-15	-25	-25	-3	-3	4.1
13	35.8	15	-34	15	-34	0	-3	1.2
13	13.2	2	-1	4	-3	0	0	-2.1
51	33.8	7	7	31	-4	0	0	-0.9
39	24.7	30	-9	28	70	0	0	-0.3
<b>50 000 C/kg</b>								
41	36.6	-45	-43	-20	-25	-1	-2	1.2
38	36.6	-6	-7	-2	-4	-2	-2	0.0
21	34.7	-6	-9	-6	-9	-1	-1	-0.4
32	29	-11	-8	0	4	-1	-1	0.6
	17.8	-8	-14	-14	-20	0	-3	2.0
8	20.8	-9	-12	-12	-13	-1	-2	3.1
<b>100 000 C/kg</b>								
24	16.7	-34	-28	-34	-27	-1	-1	-0.5
20	22.9	-10	-10	-10	-10	-1	-1	-0.3
40	45.8	-7	-7	0	0	-2	-2	-0.4
47	27.3	-3	7	-1	9	-1	-2	-0.6
67	10.2	-21	-18	-5	23	-2	0	2.8
45	33.6	31	17	-2	-1.5	0	0	-2.0
<b>500 000 C/kg</b>								
6	16.8	-24	-23	-24	-23	-1	-1	-0.3
38	27.4	-11	-11	-8	-8	-3	-3	0.8
31	20.9	24	24	0	2	0	0	0.0
37	15.2					-2	-2	2.2
28	24.9	-43	-45	0	0	0	0	-0.6
28	29.3	-14	-28	-28	-35	-1	-2	1.3

WBC indicates white blood cell; ETT, exercise tolerance test; and C/kg, CD34<sup>+</sup> cells per kilogram of body weight dose administered by intramyocardial injection. \*Days 4 and 5 of mobilization.

difference score was improved in both treatment groups at both time points, with a slightly greater improvement noted in the cell- versus placebo-treated patients at both time points ( $-1.5 \pm 1.6$  in placebo versus  $-2.4 \pm 4.4$  in CD34<sup>+</sup> cell-treated patients at 3 months and  $-0.7 \pm 2.0$  versus  $-2.3 \pm 3.1$  at 6 months; Figure 2). In contrast, the visually estimated summed difference score revealed improvements compared with baseline in both treatment groups and a slightly greater improvement in CD34<sup>+</sup> cell-treated patients at 3 months ( $-0.8 \pm 1.5$  placebo versus  $-2.5 \pm 3.3$  treated), with this trend being reversed at 6 months ( $-2.2 \pm 3.4$  versus  $-1.5 \pm 4.1$ ; Figure 2).

#### Quality-of-Life Testing

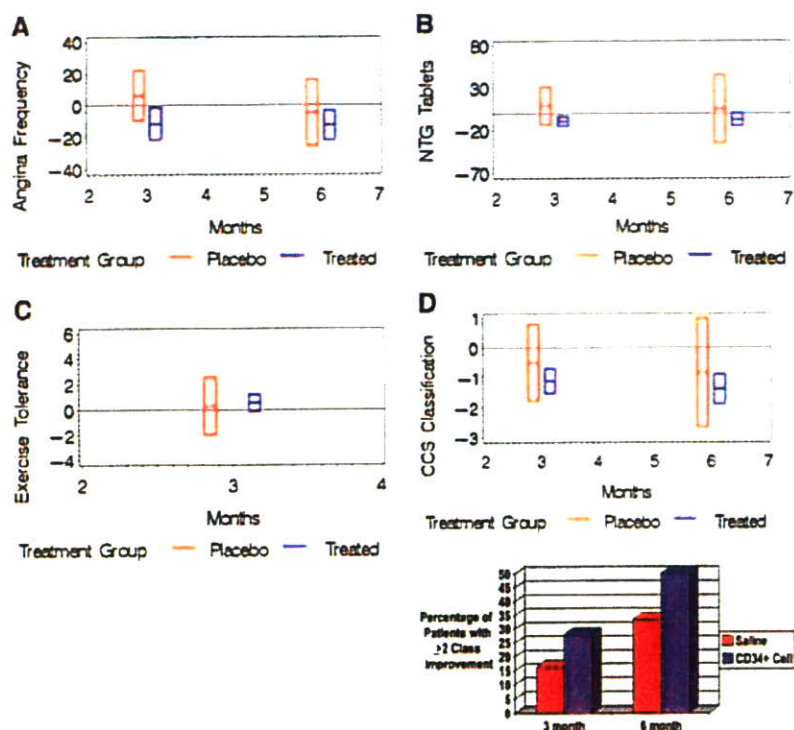
Seattle Angina Questionnaire assessment of physical limitation, angina stability, angina frequency, disease perception, and treatment satisfaction revealed improvements in both treatment groups (Figure 3). At 3 months, all 5 parameters favored the CD34<sup>+</sup> cell-treated patients, and at 6 months, 4 of

5 parameters showed trends favoring the cell- versus placebo-treated patients compared with baseline.

#### Discussion

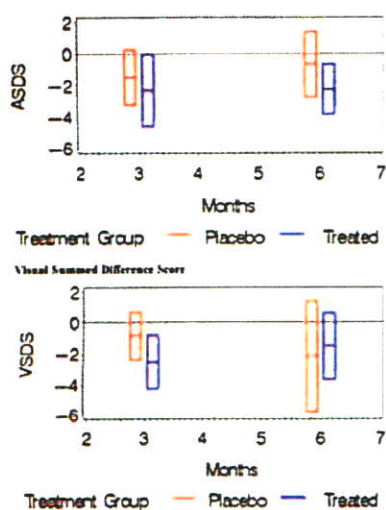
This double-blind, placebo-controlled trial of intramyocardial injection of autologous CD34<sup>+</sup> stem cells in patients with intractable angina provides preliminary evidence for the safety of this approach.<sup>10</sup> Evaluation of bioactivity reveals trends in favor of the cell-treated patient in most of the outcome measures. Together, these outcomes describe a successful first-in-human study and serve as the basis for proceeding with a larger phase IIb study, which is under way. The findings also raise many questions, most of which cannot be answered but which are nevertheless important to pose as the field of stem cell therapy continues to evolve.

Two parameters appeared worse in the placebo group at the 3-month follow-up visit: angina frequency and NTG use. At 6 months, angina frequency in the placebo-treated patients was reduced compared with baseline, whereas NTG use



**Figure 1.** Clinical symptomatic end points. All data are shown as means and 95% confidence limits. A, Angina frequency: patient-reported frequency of angina, at baseline and 3 and 6 months after injection of placebo (red) or CD34<sup>+</sup> cells (blue). B, NTG consumption: tablets per week, at baseline and 3 and 6 months after injection of placebo (red) or CD34<sup>+</sup> cells (blue). C, Exercise tolerance: total exercise time on the standard Bruce protocol in placebo (red)-injected or CD34<sup>+</sup> cell (blue)-injected patients. D, CCS angina classification. Top, CCS class at baseline and 3 and 6 months after injection. Bottom, Percentage of patients with a  $\geq 2$  class improvement compared with baseline.

remained higher. In contrast, exercise tolerance, CCS class, and the Seattle Angina Questionnaire parameters of angina frequency, physical limitation, disease perception, and treatment satisfaction all improved in the placebo group at 3 months. Taking into account the small sample size, we must be vigilant for the possibility that placebo patients may experience an exacerbation in symptoms during the study, although most of the evidence suggests a positive placebo effect. The placebo protocol involves administration of GCSF and intramyocardial injection of a solution containing autologous serum, either of which could present theoretical risks.<sup>9</sup>

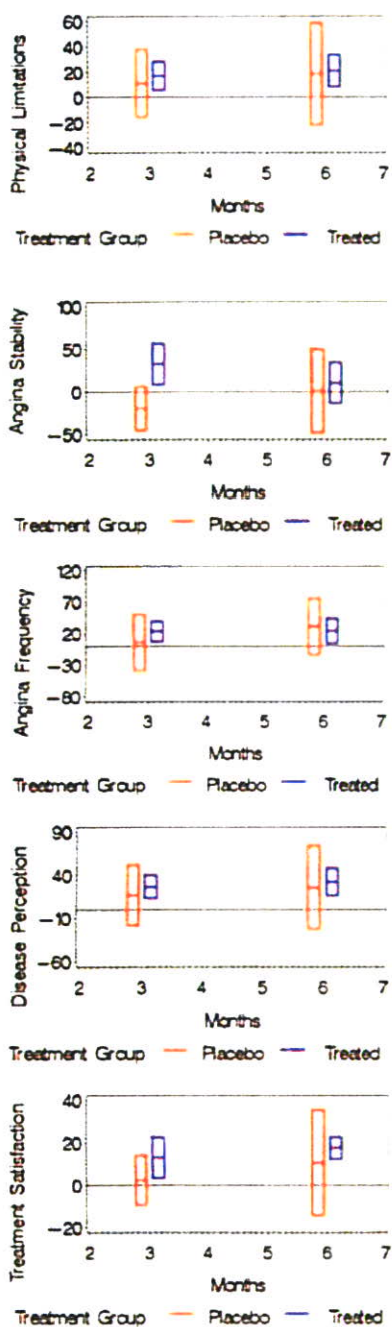


**Figure 2.** SPECT perfusion imaging. All data are shown as means with 95% confidence limits. Top, Change in automated summed difference score (ASDS) from baseline to 3- and 6-month follow-up. Bottom, Change in visually estimated summed difference score (VSDS) from baseline to 3- and 6-month follow-up.

Two fundamental questions must be addressed: What is the mechanism of the disease, and what is the proposed mechanism of CD34<sup>+</sup> stem cell therapy? The answer to the first question is critical before answering the second question and is not as self-evident as it might initially seem. Epicardial coronary disease is the underlying disease process in the target patient population; however, progressive angina and heart failure are known to occur in patients despite no apparent change in the epicardial coronary anatomy. Mounting evidence, both old and new, suggests that attrition of the myocardial microvasculature occurs progressively in the ischemic myocardium, which compounds the loss of macrovascular supply, and that interventions that protect or restore microvascular integrity can improve perfusion and function without altering the macrovascular circulation.<sup>11-16</sup> Data from preclinical models indicate that CD34<sup>+</sup> cells restore the microcirculation and improve myocardial tissue perfusion and do so despite the permanent occlusion of the epicardial vessel.<sup>5,6</sup>

The next question about the mechanism of cell therapy revolves around whether the cells participate in new vessel formation or induce neovascularization by elements within the tissue and circulating blood via paracrine effects. The literature and data from our laboratory provide evidence for both phenomena.<sup>17</sup> Most interesting among these data are the evidence that the endothelial progenitor cell (EPC) has a phenotype that drives its potency for neovascularization.<sup>18,19</sup> These data indicate that cells from patients with severe cardiovascular disease are not as functional as those collected from healthy volunteers. These findings have several implications. Clinicians will focus on identifying the specifics of the cell phenotype that define potency, thereby providing the opportunity to enhance outcome in cell-based strategies. Preclinical studies have already revealed the potential of this





**Figure 3.** Seattle Angina Questionnaire assessment: change from baseline at 3 and 6 months for the Seattle Angina Questionnaire assessment of physical limitations, angina stability, angina frequency, disease perception, and treatment satisfaction. An increased score indicates functional improvement. Data are represented as means with 95% confidence limits.

strategy.<sup>20</sup> The broader scientific appeal of the link between the EPC and endothelial function is the access that these circulating cells provide to a virtually unattainable material: human endothelium. Multiple investigators have already taken advantage of the EPC as a surrogate for the human endothelium, making observations linking the kinetics and biology of these cells with important cardiovascular disease risk factors and outcome. It is probably only a matter of time

before an EPC-based metric will be used as part of cardiovascular disease risk profiling.

The choice of the CD34<sup>+</sup> for this therapy was based on extensive preclinical data indicating that surface expression of CD34 identified a population of cells with enhanced potency for neovascularization of ischemic tissue.<sup>3,6,21</sup> Other methods of enriching the population of EPCs have been established, most notably the use of cell culture, with efficacy shown in preclinical studies and more recently in clinical trials.<sup>4,22–24</sup> We chose CD34<sup>+</sup> selection on the basis of our preclinical data that indicated enhanced efficacy and safety with CD34<sup>+</sup> versus unselected cells<sup>5,6</sup> and because a device was commercially available that could be used to purify the CD34<sup>+</sup> cells from patients, thereby obviating the requirement for a current Good Manufacturing Practices facility at each treatment site. Accordingly, the availability of an autologous stem cell with potency for therapeutic neovascularization that could be produced practically at any hospital indicated that this strategy, if successful, could be applied practically on a large scale. We considered this last feature (ie, broad applicability), to be a key feature before considering the initiation of clinical trials. In addition, the possibility that mobilization via administration of G-CSF could have an independent therapeutic effect must be considered, although G-CSF alone did not improve perfusion in preclinical models.<sup>25</sup> This possibility, along with the desire to maintain study blinding, was part of the reason that G-CSF was administered to the control population. A relationship was not detected between the degree of CD34<sup>+</sup> cell mobilization and any outcome measures; however, this possibility must be considered as studies with larger sample sizes are performed.

The selection of doses of CD34<sup>+</sup> cells was based on the results of preclinical studies.<sup>6</sup> The lowest dose was not optimally therapeutic in the animal studies and was chosen as the starting dose in this first-in-human study to begin to establish a safety profile in a conservative manner. The higher doses represented the doses at which the benefit in the animal models appeared to plateau (ie, higher doses did not provide evidence of enhanced benefit). In our pilot study, no dose-response effect was observed. Setting aside for the moment the obvious fact that the study was not powered to detect a dose response, a discussion of dosing is important. Preclinical models from which data substantiating safety and efficacy are derived are performed in young, healthy animals in which the disease is induced and treated in the course of weeks. Thus, although it is a necessary guidepost for the initiation of clinical trials, the calculation of dose must be reexamined continuously in the context of the patient population. It appears unlikely that a single administration of any dose of cells will completely reverse a disease process that has progressed over the course of decades. A single administration is a necessary starting point for a safety evaluation, but it appears likely to be replaced by an incremental approach if single administration provides evidence of partial improvement.

The present study approaches symptom relief in a patient population for whom a successful therapy has not yet been developed. Accordingly, no precedent exists on which to base clinical trial design. A significant reduction in symptoms in

treated versus control patients is imperative, but thus far, symptom relief has not been sufficient for approval of antianginal therapies. This philosophy is applied inconsistently (analgesics are routinely approved on the basis of relief of pain) and perhaps must be challenged as the population with severe coronary disease continues to expand. Nevertheless, another measure of efficacy, providing evidence of a biological activity, would be desirable. Exercise testing has been widely used as a surrogate for symptomatic improvement in studies of antianginal therapies; however, several characteristics distinguish these prior studies and investigations of the intractable angina population. Antianginal medications were developed in the preangioplasty era and were therefore tested in a younger, healthier population, many of whom had single-vessel disease. The target population of intractable angina therapies is older and has a preponderance of multivessel disease, prior myocardial infarction, and prior (sometimes multiple) bypass surgeries. Accordingly, the ability to increase exercise performance in this population may be restricted by other factors, which limits the potential utility of the exercise tolerance test. In addition, some prior studies in the intractable angina population have shown increases in exercise time in the placebo group of nearly 1 minute.<sup>26</sup> This fact is particularly interesting when viewed in the context of a randomized, controlled trial of PTCA versus best medical therapy.<sup>27</sup> In an unblinded study of relatively young, single-vessel disease patients, the PTCA-treated patients experienced a 90-second improvement in total exercise time compared with those randomized to the control arm.<sup>27</sup> If we factor in the placebo effect, documented to increase exercise time by 30 to 60 seconds in blinded studies, the impact of PTCA (a therapy that is applied as a standard of care to alleviate angina) on total exercise time, even in relatively healthy patients, is apparently quite modest.

SPECT imaging would appear to be a logical candidate to provide objective evidence for neovascularization. Our expectations in this regard are tempered, however, by the fact that SPECT imaging has been validated primarily for detection of epicardial disease and is particularly suited for detecting gradients in perfusion that result from disease of 1 or 2 vessels. Thus, although SPECT imaging, as a standard clinical modality, is being applied in most clinical trials of neovascularization, the tool is being scrutinized to determine its suitability for accurately assessing outcome.

Both positron emission tomography-computed tomography and magnetic resonance imaging offer theoretical advantages for assessment of perfusion and function but remain experimental in these applications. In addition, we are hopeful that molecular imaging techniques, capable, for example, of noninvasively quantifying new vessel formation, may offer the precise assessment of a meaningful biological end point that would enable confident assessment of our attempts at microvascular regeneration.<sup>28</sup>

Recently, published studies by Assmus<sup>23</sup> and Schachinger<sup>24</sup> have provided evidence for the therapeutic potency of cultured EPCs for the treatment of ischemic disease. The present data add to this evidence that the CD34<sup>+</sup> stem cell, isolated from the circulating blood, can be safely transplanted via intramyocardial injection and may improve perfusion and

reduce symptoms in patients with advanced coronary disease who have exhausted the currently available therapeutic armamentarium.

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### Disclosures

The study protocol was submitted to the Food and Drug Administration by Dr Losordo as the study sponsor. The data were collected and stored in a database at Caritas St. Elizabeth's Medical Center, and the investigators had full access to the data at all times. Baxter assisted with analysis of the locked data set. Drs Losordo, Henry, and Schatz are consultants to Baxter. Ken Story is an employee of Baxter Healthcare.

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### CLINICAL PERSPECTIVE

This report details a first-in-human experience with intramyocardial, transendocardial transplantation of autologous CD34<sup>+</sup> cells for intractable angina. The study design was based on preclinical data that provided evidence that selected CD34<sup>+</sup> cells were safer and more effective for revascularization of ischemic myocardium. The target population in this study, with a condition estimated to have an annual incidence in the United States of 150 000 to 250 000 per year, included patients with class III and class IV angina refractory to medical treatment and not amenable to revascularization. Features that distinguish this trial from previous reports include the fact that (1) it was performed in the United States, (2) its patients had chronic ischemia, and (3) selected stem cells collected from the peripheral circulation were used (versus bone marrow mononuclear cells in most other reports). This pilot study provided evidence for safety and feasibility and also revealed trends that favor the treatment versus control in most of the parameters assessed. As a pilot study, the trial was not powered for efficacy assessment. Together, these findings supported the initiation of a phase IIb study of 150 patients that is under way. In that study, planned for 150 patients, the lowest dose used here was not included, and the trial is powered, on the basis of the data from the present study, to detect statistically significant differences in reduction in angina.