An Open-Label Dose Escalation Study to Evaluate the Safety of Administration of Nonviral Stromal Cell-Derived Factor-1 Plasmid to Treat Symptomatic Ischemic Heart Failure


Rationale: Preclinical studies indicate that adult stem cells induce tissue repair by activating endogenous stem cells through the stromal cell-derived factor-1:chemokine receptor type 4 axis. JVS-100 is a DNA plasmid encoding human stromal cell-derived factor-1.

Objective: We tested in a phase 1, open-label, dose-escalation study with 12 months of follow-up in subjects with ischemic cardiomyopathy to see if JVS-100 improves clinical parameters.

Methods and Results: Seventeen subjects with ischemic cardiomyopathy, New York Heart Association class III heart failure, with an ejection fraction ≤40% on stable medical therapy, were enrolled to receive 5, 15, or 30 mg of JVS-100 via endomyocardial injection. The primary end points for safety and efficacy were at 1 and 4 months, respectively. The primary safety end point was a major adverse cardiac event. Efficacy end points were change in quality of life, New York Heart Association class, 6-minute walk distance, single photon emission computed tomography, N-terminal pro-brain natriuretic peptide, and echocardiography at 4 and 12 months. The primary safety end point was met. At 4 months, all of the cohorts demonstrated improvements in 6-minute walk distance, quality of life, and New York Heart Association class. Subjects in the 15- and 30-mg dose groups exhibited improvements in 6-minute walk distance (15 mg: median [range]: 41 minutes [3–61 minutes]; 30 mg: 31 minutes [22–74 minutes]) and quality of life (15 mg: –16 points [+1 to –32 points]; 30 mg: –24 points [+17 to –38 points]) over baseline. At 12 months, improvements in symptoms were maintained.

Conclusions: These data highlight the importance of defining the molecular mechanisms of stem cell-based tissue repair and suggest that overexpression of stromal cell-derived factor-1 via gene therapy is a strategy for improving heart failure symptoms in patients with ischemic cardiomyopathy. (Circ Res. 2013;112:816-825.)

Key Words: chemokine ■ chronic heart failure ■ clinical trial ■ gene transfer ■ stem cell

Chronic heart failure (CHF) is one of the leading causes of morbidity and mortality in Westernized countries, with a US prevalence of 5.8 mol/L and an incidence of 690 000 new cases per year.1 The estimated direct and indirect cost of CHF in the United States for 2008 was $34.8 billion,2 largely because of the recurrent, lengthy hospitalizations associated with the disease. A significant cause of CHF is ischemic heart disease. It has been estimated that 67% of patients with systolic CHF have CHF caused by previous acute myocardial infarction.3 Novel effective CHF treatments that improve quality of life, stabilize or improve cardiac function, and, thus, reduce the number of CHF hospitalizations will provide both clinical benefit and savings to the health-care system.

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Recently, both CHF of ischemic etiology and its precursor, acute myocardial infarction, have been targeted for treatment by stem cell-based regenerative medicine with promising early results.4 Regenerative medicine has a high therapeutic potential for treatment of ischemic cardiac disease because, unlike current treatments that focus on either alleviating symptoms or reducing cardiac workload, regenerative medicine provides an opportunity to repair and retain function in injured organs. Preliminary efficacy (ie, improvement in cardiac function) has been shown in a number of clinical trials5–7 but not all.8,9

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From the Summa Cardiovascular Institute, Summa Health System, Akron, OH (M.S.P.); Skirball Laboratory for Cardiovascular Cellular Therapeutics, Northeast Ohio Medical University, Rootstown, OH (M.S.P.); Center for Therapeutic Angiogenesis, Birmingham, AL (F.O.M.); Rush University Medical Center, Chicago, IL (G.L.S.); Columbia University Medical Center, New York, NY (W.S., M.F.); Juventas Therapeutics, Cleveland, OH (J.P., R.C., R.A.); BioCardia, Inc, San Carlos, CA (D.W.L.); Northwestern Memorial Hospital, Chicago, IL (D.W.L.); and Northwestern University Feinberg School of Medicine, Chicago, IL (D.W.L.).

Correspondence to Marc S. Penn, Summa Cardiovascular Institute, Summa Health System, Skirball Laboratory for Cardiovascular Cellular Therapeutics, Department of Integrative Medical Sciences, Northeast Ohio Medical University, Akron, OH 44304. E-mail mpeenn2@neomed.edu

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The goal of our group is to define the molecular mechanisms associated with stem cell-based repair of the heart with the goal to define therapeutic strategies that are mechanically based. Several years ago we hypothesized that stem cell-based repair of the heart is a natural process but clinically inefficient because of downregulation of key regulators of the process.\textsuperscript{10,11} We proposed that this approach could lead to the development of direct and clinically feasible regenerative therapies without the need to harvest or inject stem cells. To that end, we demonstrated previously in preclinical studies a critical role for the stromal cell-derived factor (SDF) 1: chemokine receptor type (CXCR) 4 axis in tissue repair.\textsuperscript{10,12–14} SDF-1 (CXCL12) is a naturally occurring chemokine that is rapidly and transiently upregulated in response to tissue injury.\textsuperscript{10,15} SDF-1 is a strong chemoattractant of endogenous organ-specific and bone marrow-derived stem cells and progenitor cells to the site of tissue damage, which promotes tissue preservation and blood vessel development.\textsuperscript{16} SDF-1 expression is increased in the myocardium after an infarction, but expression lasts for less than a week and, therefore, the induced stem cell homing response quickly fades.\textsuperscript{10} The short duration of SDF-1 expression reduces the potential for tissue repair. This suggests that therapeutic interventions that re-establish and prolong the ability of SDF-1 to stimulate the stem cell homing process may be beneficial for patients who have damaged heart tissue.\textsuperscript{17} Since our early observations, several groups using multiple delivery strategies have demonstrated the therapeutic potential of prolonging or re-establishing SDF-1 expression to prevent or treat cardiac dysfunction.\textsuperscript{18–21} Furthermore, it has been demonstrated recently that delivery of endogenous stem cells, like cardiac stem cells, that are activated by SDF-1 has beneficial effects.\textsuperscript{13,22,23} Taken together, these published data from multiple laboratories demonstrate that overexpression of SDF-1 has the potential to provide clinical benefit in cardiovascular ischemic disease.

We developed JVS-100, a nonviral, naked DNA plasmid encoding human SDF-1, for treatment of ischemic cardiovascular disease\textsuperscript{24} to test our hypothesis that re-establishing molecular signaling for stem cell-based repair is sufficient to induce tissue repair. Recent work in our laboratory has demonstrated the potential for direct injection of human SDF-1 plasmid into the border zone of the chronically remodeled myocardium in rats and pigs to improve cardiac function and cardiac vascular density.\textsuperscript{24,25} Furthermore, in a porcine model of heart failure delivery of JVS-100 with an endomyocardial injection catheter (BioCardia Helical Infusion Catheter, BioCardia, Inc, San Carlos, CA) demonstrated safety at doses ≤100 mg while improving cardiac function and vasculogenesis ≤90 days postinjection at doses of 7.5 and 30.0 mg.\textsuperscript{25} The ability of SDF-1 to positively impact stem cell homing, reduce programmed cell death, and promote revascularization makes nonviral SDF-1 gene transfer a promising candidate for treating ischemic cardiac disease.

We now report the findings of a completed phase 1 dose-escalation study to assess the initial safety and efficacy of increasing doses of JVS-100 delivered via an endomyocardial injection catheter to treat CHF in patients with ischemic cardiomyopathy. In each cohort, subjects received a single dose of JVS-100 via 15 endomyocardial injections. Safety was tracked at each dose by documenting all of the adverse events, with the primary safety end point being the number of major adverse cardiac events at 30 days. Therapeutic efficacy was evaluated by measuring the impact on cardiac function via standard echocardiography measurements, cardiac perfusion via single photon emission computed tomography imaging, New York Heart Association (NYHA) class, 6-minute walk distance (MWD), N-terminal pro-brain natriuretic peptide (NT-proBNP), and quality of life at 4 and 12 months.

### Methods

#### Patient Population

Subjects with heart failure attributed to previous myocardial infarction were enrolled, with the primary inclusion criteria being NYHA class III, ischemic cardiomyopathy without an acute coronary syndrome within the last 6 months, residual region of left ventricular (LV) dysfunction defined as ≥2 consecutive segments of abnormal wall motion by echocardiography, LV ejection fraction ≤40%, and an implanted functional automated implantable cardioverter defibrillator. All of the patients were required to be on optimal pharmacological therapy defined as angiotensin-converting enzyme inhibitor or angiotensin receptor blocker and β-blocker treatment for 90 days unless contraindicated. Subjects were prescribed an aldosterone inhibitor at physician discretion. Primary exclusion criteria were wall thickness <0.5 cm in any segment of the left ventricle, candidate for revascularization, epi- dermal growth factor receptor <30 mL/min, a history of aortic valve regurgitation >2, moderate-severe aortic stenosis (<1.5 cm²), aortic aneurysm >3.8 cm, any history of cancer excluding curable nonmela- noma skin malignancy, presence of LV thrombus, or persistent atrial fibrillation.

After informed consent, baseline evaluations were obtained, including medical history and physical examination, ECG, echocardiology, routine blood tests including hematology, N-terminal pro-brain natriuretic peptide and blood chemistry, urinalysis, serum pregnancy test for all women of childbearing potential, single photon emission computed tomography perfusion imaging, and urinalysis. The patient was considered enrolled when he or she entered the catheterization laboratory in preparation for the injection procedure.

#### Study Design and Injection Procedure

Seventeen subjects received 1 of 3 escalating doses of JVS-100. Dose escalation occurred by increasing the total amount of JVS-100 delivered per subject from 5 mg (low dose, n=4 subjects) to 15 mg (middle dose, n=6 subjects) to 30 mg (high dose, n=7 subjects). Before delivery of JVS-100, the infarct zones were identified using baseline echocardiography defined by the interventional cardiologist, and the peri-infarct zones were targeted for injection. Peri-infarct territories...
with LV wall thickness <0.9 cm identified by echocardiography were not injected. Each subject received fifteen 1-mL injections of JVS-100 delivered by an endomyocardial needle catheter transendocardially into the peri-infarcted region of the myocardium from a percutaneous LV approach using the BioCardia (San Carlos, CA) Helical Infusion Catheter as described previously.38

Visits and Assessments
After JVS-100 administration, subjects were monitored overnight for 18 hours (including echocardiography within 6 hours) postdose and had scheduled visits at 3 and 7 days postinjection to ensure that there were no safety concerns. All of the subjects had follow-ups at 30 days (1 month), 120 days (4 months), and 360 days (12 months) to assess safety and cardiac function. After the final dosing of each cohort, all of the safety data collected during the 14 days after each subject’s dosing with JVS-100 were reviewed by an independent data safety monitoring committee before approval for dose escalation.

Study Outcomes
The primary safety end point was the number of major adverse cardiac events occurring at the 1-month follow-up. Efficacy end points included change from baseline in left ventricular end systolic volume, LV ejection fraction, NYHA classification, quality of life (Minnesota Living With Heart Failure Questionnaire), 6MWD, NT-proBNP, and myocardial perfusion. Perfusion was determined by single photon emission computed tomography imaging and included rest and stress perfusion, as well as the summed difference score (SDS) calculated as the difference between stress and rest perfusion in each LV segment, a measurement of ongoing ischemia. Adverse events were tracked for each subject throughout the study.

Statistical Analysis
This was an open-label, nonrandomized study with relatively small sample sizes. Therefore, descriptive nonparametric statistics (median and interquartile range) were used to compare continuous efficacy variables across dosing groups. Safety parameters were collected and assessed qualitatively or summarized quantitatively by descriptive statistics. The data from each efficacy parameter were assessed at each time point as change from baseline for each patient. Ranges were reported as the 25th to 75th percentile range (ie, the end points of the interquartile range; Microsoft Excel, Microsoft, Seattle, WA). Statistical significance for changes between baseline and 4-month efficacy parameters was determined using a Wilcoxon signed-rank test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA) with a P value of <0.05 considered significant. In our preclinical porcine study, we observed no safety concerns. All of the subjects had follow-ups at 3 and 7 days postdose and the pressures were lowered pharmacologically before starting the procedure, and the pressures were lowered pharmacologically before starting the procedure.

Results
The results of this study include the 4- and 12-month safety and efficacy data from all of the cohorts.

Baseline Characteristics
Consecutive patients who qualified were enrolled from March to December 2010. Thirty-four patients were screened from which the 17 patients enrolled qualified (Figure 1, consort diagram). The majority of screen failures were attributed to wall thickness, because one of the requirements of the infusion catheter is that all of the patients enrolled could not have any region of the left ventricle with wall thickness <0.5 cm. The baseline characteristics of enrolled patients are listed in Table 1. There were no statistical differences in any parameter except the 6MWD between the combined treatment groups of 15- and 30-mg of JVS-100 at baseline. Overall, the patients did not have substantial amounts of ischemia, because the SDS at baseline in each cohort was nominal. All of the subjects were on stable optimal medical therapy. The 4 enrolled subjects not receiving angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and 1 not receiving β-blocker therapy were documented to have contraindications for these medications. One NYHA class II patient who met all of the other inclusion/exclusion criteria was granted a waiver for enrollment because his 6MWD was <400 m (280 m), which has been shown to be a predictor of poor cardiovascular clinical outcomes independent of NYHA class.27

Safety of JVS-100
All of the subjects received the intended number of 15 intracardiac injections of JVS-100. Of the 17 subjects enrolled, 15 are alive. All 17 of the patients survived to 228 days. All 15 of the surviving patients have completed the intended 12-month follow-up. The primary safety end point of major adverse cardiac events at 1 month postinjection was met, with only 3 major adverse cardiac events occurring within 1 month of dosing. The subject who experienced CHF decompensation at day 16 ultimately died because of worsening failure to thrive. The subject who had acute on chronic CHF decompensation at day 0 had unexpectedly high end diastolic pressures immediately before the procedure, and the pressures were lowered pharmacologically before starting the procedure.

Through the 12-month follow-up, 26 serious adverse events (SAEs) were reported in 8 subjects (Table 2). Nine subjects had no SAEs. The majority of SAEs were related to underlying disease, with no SAE deemed likely related to the drug. The 1 unanticipated SAE, systemic lupus erythematosus flare, occurred in a subject who, in retrospect, was found to be aninuclear antibody–double-stranded DNA positive before the time of enrollment without a history of systemic lupus erythematosus. The subject was treated with prednisone,
and the symptoms resolved within 45 days. The 2 deaths that occurred were because of unwitnessed cardiac arrest (28 days postdosing) and worsening failure to thrive (46 days postdosing) in a patient who experienced multiple concomitant diseases, including depression, diabetes mellitus, and critical limb ischemia. Neither death was related to study drug, study procedure, or study device. This survival rate (88%) in this small population is consistent or better than expected for this procedure, or study device. This survival rate (88%) in this small population is consistent or better than expected for this

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Full Trial (n=17)</th>
<th>5 mg (n=6)</th>
<th>15 mg (n=6)</th>
<th>30 mg (n=7)</th>
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</thead>
<tbody>
<tr>
<td>JVS-100 dose, mg</td>
<td></td>
<td>5</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Age, y</td>
<td>65.9 (62.4, 71.1)</td>
<td>70.1 (67.7, 72.9)</td>
<td>62.5 (59.5, 68.4)</td>
<td>65.9 (62.7, 72.3)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>12/5</td>
<td>4/0</td>
<td>4/2</td>
<td>4/3</td>
</tr>
<tr>
<td>6-min walk, m*</td>
<td>280 (225, 344)</td>
<td>315 (261, 390)</td>
<td>338 (321, 376)**</td>
<td>225 (207, 258)**</td>
</tr>
<tr>
<td>Biventricular pacing, %</td>
<td>4 (23.5)</td>
<td>2 (50)</td>
<td>1 (16.7)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Time from last MI, y</td>
<td>5.6 (3.5, 8.7)</td>
<td>9.7 (8.1, 9.8)</td>
<td>4.8 (2.7, 8.1)</td>
<td>3.85 (3.3, 5)</td>
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<tr>
<td>Quality-of-life score</td>
<td>59 (42, 70)</td>
<td>52 (49.5, 55.3)</td>
<td>69.5 (33.3, 75)</td>
<td>59 (50, 66.5)</td>
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<tr>
<td>LVESV, mL</td>
<td>112 (81.5, 129.8)</td>
<td>97 (78.8, 130.8)</td>
<td>128 (125.5, 131.3)</td>
<td>82.5 (75.5, 106.8)</td>
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<tr>
<td>LVEF, %</td>
<td>33.5 (28.3, 36.3)</td>
<td>36.5 (34.3, 37.5)</td>
<td>29.5 (26, 33.8)</td>
<td>34 (30, 38.8)</td>
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<tr>
<td>SRS</td>
<td>25 (21, 34)</td>
<td>26 (24, 27.8)</td>
<td>25 (23.5, 31.8)</td>
<td>23 (17, 38.5)</td>
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<td>SSS</td>
<td>27 (24, 38)</td>
<td>28 (25.5, 31.8)</td>
<td>28.5 (23.8, 38.5)</td>
<td>27 (22, 39.5)</td>
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<td>SDS</td>
<td>2 (0.7)</td>
<td>2.5 (0.3, 6.3)</td>
<td>3.5 (0.3, 6.8)</td>
<td>2 (0, 5.5)</td>
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<td>ACE inhibitor or ARB, %</td>
<td>13 (77)</td>
<td>4 (100)</td>
<td>6 (100)</td>
<td>5 (71)</td>
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<td>β-Blocker, %</td>
<td>16 (94)</td>
<td>4 (100)</td>
<td>6 (100)</td>
<td>6 (86)</td>
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<td>Statin, %</td>
<td>17 (100)</td>
<td>4 (100)</td>
<td>6 (100)</td>
<td>7 (100)</td>
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<tr>
<td>ASA, %</td>
<td>15 (88)</td>
<td>4 (100)</td>
<td>6 (100)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>9 (53)</td>
<td>2 (50)</td>
<td>4 (67)</td>
<td>3 (43)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ASA, aspirin or acetylsalicylic acid; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; M/F, male/female; MI, myocardial infarction; SRS, summed difference score; SSS, summed stress score. Data are presented as median (25th percentile, 75th percentile) or n (%), unless otherwise specified.

*P=0.036 for difference in medians by Kruskal-Wallis test.

**Significant difference was calculated by the Dunn multiple comparison test (P<0.05) for comparison of cohort 2 vs cohort 3.

efficacy: JVS-100 Provides Functional Benefit at 4 and 12 Months Postdosing

The primary efficacy data at 4 months after injection from the low (5 mg), middle (15 mg), and high doses (30 mg) suggest that subjects are exhibiting improvement in functional status and stabilization of ventricular function at 4 months postdosing compared with baseline. The 4- and 12-month improvements in 6MWD are summarized in Figure 2A. These data demonstrate a significant increase at 4 months in the 15- and 30-mg dose cohorts (median change, 41 and 31 m, respectively), with a statistically significant increase in the combined 15- and 30-mg treatment group (P<0.002). At 12 months after treatment, there was continued improvement in the median 6MWD in the 30-mg dose and combined 15- and 30-mg dosing groups, but a reversion to baseline in 6MWD in the 5- and 15-mg treatment groups.

The data in Figure 2B show changes in Minnesota Living With Heart Failure Questionnaire (MLWHFQ) scores at 4 and 12 months after treatment relative to baseline. Subjects in the combined middle- (15 mg) and high-dose (30 mg) groups had 10.0- and 19.5-point greater improvements compared with the low-dose (5 mg) group at 4 and 12 months, respectively. Patients in the higher dose groups demonstrated robust improvements from baseline in quality-of-life score (median change, –16 and –24 points; Figure 2B). The combined 15- and 30-mg treatment groups exhibited a statistically significant improvement in MLWHFQ (P<0.05). Importantly, the improvement in MLWHFQ seen at 4 months was sustained at 12 months in the 15- and 30-mg treatment groups (P<0.01). It is interesting to note that, at 12 months, there was a worsening of quality of life (7.5-point increase in MLWHFQ) in the low-dose, 5-mg treatment group.

Assessment of cardiac function via echocardiography shows similar dose-dependent trends (Figure 3) at 4 and 12 months after treatment. There were nonstatistically significant small levels of decline (decreased ejection fraction; increased LV end systolic volume) in cardiac function in all of the treatment groups at 12 months after treatment. It is interesting to note that there was a trend with less decline in function with increasing dose of JVS-100 (P value for change in function between baseline at 12 months was 0.37, 0.74, and 0.73, for 5, 15, and 30 mg, respectively; Table 3 and Figure 3).
All of the patients enrolled had an elevated NT-proBNP at baseline (Figure 4). Consistent with improvements in 6MWD in the 15- and 30-mg groups at 4 months we observed a trend toward a decrease in NT-proBNP at 4 months. Interestingly, the average NT-proBNP remained less than baseline in the 30-mg but not the 15-mg dose group at 12 months. This parallels our findings with 6MWD at 12 months as well.

Our data show that JVS-100 led to improvements in 6MWD and MLWHFQ with stabilization of ventricular function. The combination of effects appears to have led to improvement in overall CHF status. A higher percentage of patients improved ≥1 NYHA class in the 15-mg group (40%) and 30-mg groups (50%) compared with the 5-mg group (25%; Figure 5). Importantly, no patient in any group worsened NYHA class at 4 months. When data from the 15- and 30-mg groups were combined, we observed a statistically significant improvement compared with baseline in 6MWD ($P=0.002$), quality of life ($P<0.05$), and average NYHA class ($P<0.05$). There was

<table>
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<th>Subject Number</th>
<th>SAE Term</th>
<th>Time, d</th>
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<th>Relationship: Study Procedures</th>
<th>Relationship: Underlying Disease</th>
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<td>335</td>
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CHF indicates chronic heart failure; NSAIDs, nonsteroidal anti-inflammatory drugs; Pt, patient; SAE, serious adverse event; and SOB, shortness of breath.

Table 2. Summary of SAEs Through 12 Months

CHF indicates chronic heart failure; NSAIDs, nonsteroidal anti-inflammatory drugs; Pt, patient; SAE, serious adverse event; and SOB, shortness of breath.
no loss of improvement in NYHA class at 12 months after treatment. In fact, we observed additional patients improving 2 NYHA classes with 3 patients at class I at the end of the trial (none of these were the 1 class II patient enrolled into the trial).

The SDS measured the extent of reversible ischemic tissue in each patient. At baseline, patients did not show reversible ischemia, because the SDS baseline values were low for each cohort (Table 1). The effect of JVS-100 on SDS by cohort continues to demonstrate a dose-dependent effect. There was an increase in SDS from baseline to 4 months in the 5-mg group (median increase, 8.5 points [interquartile range, 5.0–9.0]), whereas SDS stabilized in the 15- and 30-mg groups (15 mg: median, 0.0 [interquartile range, −3.5 to 5.0]; 30 mg: median, 0.0 [interquartile range, −1.5 to 1.0]). When the change in SDS in the 5-mg group was compared with the combined 15- and 30-mg treatment group, the difference was statistically significant (P<0.01).

In summary, at 4 and 12 months postdosing, the maintenance of perfusion, heart size, and ventricular function, coupled with improvement in clinical parameters including 6MWD and quality of life, demonstrate that the patients treated in this trial appear to be improving relative to baseline at all of the doses, with more robust effects in the 15- and 30-mg dose groups.

**Discussion**

Stem cell-based repair of injured tissue has garnered significant attention in the scientific, clinical, and lay press over the past decade. Progress in the field has identified several novel stem cell sources that have yielded exciting and promising results that could significantly improve patient outcomes in the years ahead. Perhaps just as importantly, scientific investigation into the mechanisms of action of stem cell repair has led to many discoveries of novel or new functions of previously defined paracrine factors that could be exploited as therapeutic targets. One example of this emerging therapeutic paradigm is our work and that of others defining the critical role of the SDF-1:CXCR4 axis in tissue repair.30,20,30–31

The SDF-1:CXCR4 axis has been shown to be critical in tissue repair in multiple organ systems, including the eye, heart, kidney, liver, brain, and skin.30,33–36 Specific to the heart, the SDF-1:CXCR4 axis has been shown to be critical for cardiogenesis.37,38 Our interest is to determine whether the effects of stem cell-based tissue repair can be induced through careful manipulation of the molecular signals that regulate...
the process. Given the failure to demonstrate clinical benefit in recent cell therapy trials, we further hypothesize that, by moving the therapy closer to the mechanism of action, the field may generate more robust clinical responses.

In this study we assessed the safety and potential therapeutic role of such an approach in the treatment of CHF. Recent advances in cellular transplantation, which have focused on the use of patient-derived cardiac or bone marrow-derived stem cells, have demonstrated that induction of stem-cell mobilization and recruitment of these stem cells to the infarct area result in reverse remodeling of the heart, increased cardiac output, and healthier patients. It has been hypothesized that much of the benefit from cell therapy is derived from stem cell release of paracrine factors, such as SDF-1, that orchestrate the natural repair process. The beneficial effects of SDF-1 treatment are well characterized in cardiovascular models of ischemia and hypoxic stress. The data in this study now extend the potential importance of the SDF-1:CXCR4 axis to clinical populations.

The dose-dependent effects of human SDF-1 plasmid on efficacy are consistent with our preclinical data and suggest that 5 mg is a suboptimal therapeutic dose. In general, the 15- and 30-mg dose groups show more substantial benefit in clinical parameters (Figures 2 and 5) compared with the 5-mg dose at 4 and 12 months after treatment. Importantly, the SDS score of our population demonstrates that this patient population did not harbor significant ischemia at baseline but rather evidence of peri-infarct/border zone ischemia. The fact that the 15- and 30-mg groups have no residual ischemia at 4 months suggests that the transient overexpression of SDF-1 leads to an increase in perfusion at the infarct border zone, which is consistent with all of the preclinical studies to date.

The trends in improvement observed at 4 and 12 months in clinical parameters in the middle- and high-dose groups observed in this study are consistent with therapeutic benefit demonstrated in a recent regenerative medicine heart failure trial where autologous bone marrow cells were delivered to ischemic cardiomyopathy patients. The 31- to 41-point improvement in 6MWD and 16- to 24-point improvement in quality of life (Figure 2A) are similar to improvements in previous heart failure studies that have demonstrated therapeutic benefit, including improvement in morbidity and mortality. The improvements also are comparable to early trials investigating biventricular pacing efficacy, which ultimately demonstrated improvements in heart failure symptoms, morbidity, and mortality in large randomized trials. Importantly heart failure symptoms, such as 6MWD, have correlated well with survival

<table>
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<th>12 months</th>
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<td></td>
<td>91±13</td>
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EF indicates ejection fraction; and LVESF, left ventricular end systolic volume.

Figure 4. Effects of JVS-100 on N-terminal pro-brain natriuretic peptide (NT-proBNP) levels at 4 and 12 months after treatment. Data show individual NT-proBNP levels in each patient at baseline, 4 and 12 months after treatment. Open circles represent baseline data, grey and black filled circles represent levels at 4 and 12 months after treatment, respectively. Black lines represent mean for each dosing group at each time point. There were no statistically significant differences between NT-proBNP levels within a dosing group over time.

Figure 5. JVS-100 improves New York Heart Association (NYHA) class at 4 and 12 months. Data depict the percentage of patients with NYHA class I, II, and III chronic heart failure (CHF) at baseline and 4 and 12 months after injection in the combined 15- and 30-mg dose cohorts.
in patients with mild-to-moderate heart failure. Therefore, our nonviral SDF-1 gene therapy may have a positive impact on heart failure survival as well. This will need to be tested in larger, randomized trials.

We have overcome several challenges associated with cell-based therapy and developed a novel SDF-1 gene therapy platform to treat patients with heart failure. Nonviral gene delivery is a simple delivery method that has been tested clinically in ischemic patients for >15 years. A substantial body of literature, both preclinical and clinical, has suggested that nonviral vector delivery of therapeutic genes is safe and effective. Consistent with previous nonviral gene therapy cardiovascular trials, we demonstrated that delivering nonviral human SDF-1 plasmid after endomyocardial injection is safe and demonstrates statistically significant improvements at 4 months in a number of end points, including 6MWD, quality of life, and NYHA class.

Limitations
This was a first-in-human, open-label dose-escalation study of an invasive procedure. As such, it was not possible to include a control group. Therefore, all of the efficacy data are presented compared with each patient’s baseline values.

Conclusions
The clinical data suggest that re-establishment of SDF-1 expression via endomyocardial delivery of a nonviral vector to patients with symptomatic chronic ischemic heart failure is safe. Data from the 15- and 30-mg groups show statistically significant improvements in 6MWD, quality of life, and NYHA class over baseline at 4 months that are sustained to 12 months. These data suggest a plausible mechanism of action for stem cell-based tissue repair and demonstrate that defining the molecular signals associated with stem cell-based repair is a viable strategy to define novel targets for therapy.

Acknowledgments
We thank Dr Timothy J. Miller for his insights and tireless work, the site investigators, notably Drs Marc Krichavsky and William Cotts at Northwestern University, and the investigators’ staff, notably the following research coordinators: Susan DeRamus, Amy Graf, Sherrie Wolfe, Paula Williams, Mary Beth Marks, and Carlos Reyes-Vidal. We also thank the study participants who made this research possible. We thank Cheryl Wong Po Foo for providing catheter support in many of the injection procedures. We gratefully acknowledge the assistance of staff at the Cleveland Clinic CSResearch Imaging Core (Cleveland, OH), who performed all of the imaging analyses and training for this study.

Accelovance, Inc, was the coordinating center for the trial and provided data management, clinical laboratory, and statistical support among other clinical research organization functions. Drug Safety Solutions provided medical monitoring and pharmacovigilance for 12 months. All of the authors reviewed the article before submission. M.S.P., D.W.L., and W.S. serve on the Juventas Scientific Advisory Board and have equity in the company. Drs Pastore and Aras and Ruth Clemens are employees of Juventas Therapeutics, Inc, and have equity in the company. Dr Rouy is an employee of Biocardia, Inc, and receives salary and equity in the company. Drs Mendelsohn, Sherman, Farr, Schaer, and Losordo are investigators at enrolling sites of the clinical study at their respective institutions.

Sources of Funding
Juventas Therapeutics, Inc, funded this study. The sponsor of the study collaborated with the investigators in protocol design, data analyses, interpretation, and preparation of the report. Juventas Therapeutics, Inc, had full responsibility for the data and all analyses. The authors had full freedom to access and interpret the data. One-hundred percent of source data at each clinical site was monitored by an independent contract research organization to verify accuracy. The corresponding author (M.S.P) along with the study principal investigator (D.W.L.) had full access to the study data and had full responsibility for the decision to submit for publication.

Disclosures
Marc Penn is named as an inventor on patent applications filed by the Cleveland Clinic for the use of SDF-1 to treat cardiovascular disease that have been licensed by Juventas Therapeutics, Inc. As such, he is eligible for royalties from and equity in Juventas Therapeutics, Inc. Dr Penn is the founder and chief medical officer of Juventas Therapeutics, Inc, and as such he is a paid consultant. Drs Pastore and Aras and Ruth Clemens are employees of Juventas Therapeutics, Inc, and have equity in the company. Dr Rouy is an employee of Biocardia, Inc, and receives salary and equity in the company. Drs Mendelsohn, Sherman, Farr, Schaer, and Losordo are investigators at enrolling sites of the clinical study at their respective institutions.

References


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What Is Known?

• Stem cell delivery to the heart improves cardiac function in part through paracrine mechanisms.
• SDF-1 is the key regulator of stem cell homing in acute myocardial infarction.
• Overexpression of SDF-1 in chronic heart failure leads to neovascularization, recruitment of cardiac stem cells, and bone marrow-derived stem cells to the infarct and infarct border zone and improves cardiac function.

What New Information Does This Article Contribute?

• Delivery of an SDF-1 encoding plasmid (JVS-100) to patients with symptomatic heart failure because of ischemic cardiomyopathy improves clinical status 4 and 12 months later.
• Transient overexpression of SDF-1 in ischemic cardiomyopathy decreases peri-infarct ischemia at 4 months.
• Higher doses of DNA plasmid delivered to the myocardium than was used in previous studies is safe and may have greater efficacy than dosing implemented in previous studies.

SDF-1 is a critical factor for the recruitment of stem cells to injured tissues, as well as a critical paracrine factor released by mesenchymal stem cells for the preservation and treatment of cardiac dysfunction. In this study we tested the hypothesis that overexpression of SDF-1 in the myocardium of patients with ischemic cardiomyopathy and symptomatic CHF would lead to improvement in clinical performance at 4 and 12 months after treatment. We chose to deliver SDF-1 as a plasmid leading to SDF-1 expression for ~18 days based on previous in vitro studies. Our open-label dose-escalating study shows that delivery of 5, 15, and 30 mg of JVS-100, a DNA plasmid encoding human SDF-1, to patients with chronic heart failure is safe and that, at 4 and 12 months, patients demonstrate improved clinical status. These data suggest that the benefits of stem cell therapy may be achievable by inducing endogenous stem cell repair without the need for delivery of exogenous stem cells.

An Open-Label Dose Escalation Study to Evaluate the Safety of Administration of Nonviral Stromal Cell-Derived Factor-1 Plasmid to Treat Symptomatic Ischemic Heart Failure

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