Safety and Efficacy of Ixmyelocel-T
An Expanded, Autologous Multi-Cellular Therapy, in Dilated Cardiomyopathy

Timothy D. Henry, Jay H. Traverse, Baron L. Hammon, Cara A. East, Brian Bruckner, Ann E. Remmers, David Recker, David A. Bull, Amit N. Patel

Rationale: Ixmyelocel-T is associated with a wide range of biological activities relevant to tissue repair and regeneration.

Objective: To evaluate the safety and efficacy of ixmyelocel-T in 2 prospective randomized phase 2A Trials administered via minithoracotomy or intramyocardial catheter injections in patients with dilated cardiomyopathy (DCM) stratified by ischemic or nonischemic status.

Methods and Results: In IMPACT-DCM, patients were randomized to either ixmyelocel-T or standard-of-care control in a 3:1 ratio (n=39); ixmyelocel-T was administered intramyocardially via minithoracotomy. In Catheter-DCM, patients were randomized to either ixmyelocel-T or standard of care control in a 2:1 ratio (n=22); ixmyelocel-T was administered intramyocardially using the NOGA Myostar catheter. Only patients randomized to ixmyelocel-T underwent bone marrow aspiration and injections. In the 2 studies, a total of 61 patients were randomized, and 59 were treated or received standard of care. Fewer ischemic patients treated with ixmyelocel-T experienced a major adverse cardiovascular event during follow-up when compared with control patients. A similar benefit was not seen in the nonisemic patients. Heart failure exacerbation was the most common major adverse cardiovascular event. Ixmyelocel-T treatment was associated with improved New York Heart Association class, 6-minute walk distance, and Minnesota Living with Heart Failure Questionnaire scores in the ischemic population relative to control; a similar trend was not observed in the nonischemic population.

Conclusions: Intramyocardial injection with ixmyelocel-T reduces major adverse cardiovascular event and improves symptoms in patients with ischemic DCM but not in patients with nonischemic DCM. (Circ Res. 2014;115:730-737.)

Key Words: cardiomyopathy, dilated ■ clinical trial ■ heart failure ■ stem cell

Heart failure (HF) remains a major public health burden, affecting ≈5.1 million adults in the United States. Approximately 50% of patients diagnosed with HF will die within 5 years, and the prevalence is growing. Improvements in the pharmacological and surgical management of patients with cardiovascular disease have improved leading to increased survival, which in turn has led to an increasingly elderly patient population more likely to develop worsening, irreversible HF.1,2

Despite optimal medical therapy, ventricular assist devices and cardiac transplantation are frequently the only remaining options for these patients when medication and device therapies fail.3 Cell therapy has emerged as an attractive alternative therapy, given the positive preclinical results and encouraging early clinical trial results.4-7 Recently, the National Heart, Lung, and Blood Institute-sponsored FOCUS trial demonstrated no overall improvement in maximal oxygen consumption or end-systolic volume in 92 patients (61 treated and 31 placebo controls) with ischemic cardiomyopathy who underwent intramyocardial delivery of autologous bone marrow mononuclear cells (BMMC).8 However, a significant improvement (2.7%) in left ventricular ejection fraction (LVEF) was observed that was directly related to both cell composition (higher CD34+ or CD133 cell counts) and patient age, consistent with an observed age-related decline in the number and potency of autologous BMMCs.9,10 This has stimulated interest in alternative mechanisms to enhance the effectiveness of cell therapy, including the...
of Helsinki principles and approved by the appropriate institutional review boards. All patients gave written informed consent.

**Patient Selection**
Both studies enrolled a high-risk patient population with ischemic or nonischemic DCM based on the following criteria: World Health Organization Definitions and Classifications,

symptomatic HF, New York Heart Association (NYHA) class III or IV, LVEF ≤30%, and ineligibility for percutaneous or surgical revascularization.

Patients with ischemic DCM had a history of myocardial infarction or evidence of clinically significant (≥20% narrowing of a major epicardial artery) coronary artery disease. Patients without a history of myocardial infarction were required to have coronary angiography within the past 5 years. As part of the prescreening process, either the Aastrom medical monitor or Principle Investigators (T.D.H. and A.N.P.) reviewed available medical history jointly with the investigator to evaluate whether a patient could be screened. Eligibility for revascularization was determined by the investigational team (cardiac surgeon and interventional cardiologist).

Eligible patients were between 18 and 86 years old, taking optimal medical therapy for HF, and had an automated implantable cardioversion defibrillator unless contraindicated. In both studies, optimal medical management was defined as a stable drug treatment for the past month and no new medications within the past 3 months. Patients were excluded if they had severe valvular heart disease, history of severe chronic obstructive pulmonary disease, body mass index ≥40 kg/m², acute coronary syndrome, end-stage renal disease requiring dialysis, or substance abuse in the past 6 months. Standard exclusions for NOGA mapping or intramyocardial injection (aortic valve disease, severe aortic disease, LV thrombus, and uncontrolled atrial fibrillation) were also used in Catheter-DCM (Online Methods).

**Treatment**
Patients randomized to the ixmyelocel-T group underwent a ≥60-mL bone marrow aspiration from the posterior iliac crest during an outpatient procedure. The bone marrow aspirate was shipped overnight for manufacturing at Aastrom Biosciences. Ixmyelocel-T was produced by incubating the patient’s collected bone marrow aspirate in a proprietary bioreactor under controlled conditions and then by harvesting the expanded cell populations after 12±1 days of culture. The expanded cell population consists of mesenchymal stromal cells (CD90+) and alternately activated CD45⁺CD14⁺ autologous macrophages. All products met the following release specifications: 35 to 295×10⁶ cells with >70% cell viability; 5% to 55% of cells were macrophages. All products met the following release specifications: 35 to 295×10⁶ cells with >70% cell viability; 5% to 55% of cells were macrophages.

We report here the results of 2 phase 2A clinical trials of intramyocardial delivery of ixmyelocel-T in patients with end-stage HF because of ischemic and nonischemic dilated cardiomyopathy (DCM).

**Methods**

**Study Design**
Two prospective, randomized, open-label, multicenter, phase 2A trials were conducted to assess the safety and efficacy of ixmyelocel-T administered via minithoracotomy or intramyocardial catheter injections with the NOGA Myostar in patients with DCM stratified by ischemic or nonischemic status.

The surgical study (IMPACT-DCM; ClinicalTrials.gov Identifier: NCT01020968) was conducted from April 2010 to March 2013. Eligible patients were randomized to either ixmyelocel-T or standard of care treatment in a 3:1 ratio (n=39). The catheter study (Catheter-DCM; NCT01020968) was conducted from April 2010 to March 2013. Eligible patients were randomized to either ixmyelocel-T or standard of care treatment in a 3:1 ratio (n=22). In both studies, only patients randomized to ixmyelocel-T treatment underwent bone marrow aspiration and cell injection based on discussions with the Food and Drug Administration. Patients in the treated and control groups had clinic visits through 12 months, followed by a 24-month phone call for safety assessments. An independent Data Safety Monitoring Board met periodically to review serious adverse events. The studies were conducted in accordance with Declaration of Helsinki principles and approved by the appropriate institutional review boards. All patients gave written informed consent.

**Nonstandard Abbreviations Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>6MWD</td>
<td>6-minute walk distance based on 6-minute walk test</td>
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<td>BMMC</td>
<td>bone marrow mononuclear cells</td>
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<td>DCM</td>
<td>dilated cardiomyopathy</td>
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<td>HF</td>
<td>heart failure</td>
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<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
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<tr>
<td>MACE</td>
<td>major adverse cardiac event</td>
</tr>
<tr>
<td>MLHFQ</td>
<td>Minnesota Living with Heart Failure Questionnaire</td>
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<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
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<table>
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<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>CATH</td>
<td>Catheter-DCM</td>
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<tr>
<td>CD90</td>
<td>human stem cells</td>
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<tr>
<td>NOGA</td>
<td>navigation and guidance system</td>
</tr>
<tr>
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**Safety Monitoring Board** met periodically to review serious adverse events. An independent Data Safety Monitoring Board reviewed safety assessments. Patients in the control group received standard-of-care treatment for DCM, according to accepted medical practices. After a data review by the Data Safety Monitoring Board, patients in the control

**Ixmyelocel-T** is an expanded multicellular therapy cultured from autologous BMMC comprising mesenchymal (ie, granulocytes, monocytes, and mixed myeloid progenitors) and lymphoid cell types (ie, T cells, B cells, and mixed lymphoid progenitors) that express CD45⁺ on the cell surface, as well as CD90⁺ mesenchymal stromal cells. Within the population of CD45⁺ cells is a subpopulation of CD45⁺CD14⁺ autologous macrophages (CD14⁺Auto⁺) M2-like macrophages. Although all of these cell types are found in bone marrow, the number and proportion of CD90⁺ and CD14⁺Auto⁺ cells are significantly greater in the ixmyelocel-T product as a result of expansion during the manufacturing process. In comparison with the relatively small reservoir of these 2 cell types in bone marrow, ixmyelocel-T contains ≈200- and 50-fold the number of M2-like macrophages and mesenchymal stromal cells, respectively.

A range of biological activities relevant to tissue repair and regeneration has been demonstrated reflecting the multicellular composition of ixmyelocel-T. In addition, ixmyelocel-T was associated with improved ischemic ulcer healing and a reduction in the rate of amputation in a placebo-controlled phase 2 study in patients with critical limb ischemia. We report here the results of 2 phase 2A clinical trials of intramyocardial delivery of ixmyelocel-T in patients with end-stage HF because of ischemic and nonischemic dilated cardiomyopathy (DCM).

**Treatment**
Patients randomized to the ixmyelocel-T group underwent a ≥60-mL bone marrow aspiration from the posterior iliac crest during an outpatient procedure. The bone marrow aspirate was shipped overnight for manufacturing at Aastrom Biosciences. Ixmyelocel-T was produced by incubating the patient’s collected bone marrow aspirate in a proprietary bioreactor under controlled conditions and then by harvesting the expanded cell populations after 12±1 days of culture. The expanded cell population consists of mesenchymal stromal cells (CD90⁺) and alternately activated CD45⁺CD14⁺ autologous macrophages. All products met the following release specifications: 35 to 295×10⁶ cells with >70% cell viability; 5% to 55% of cells were macrophages. All products met the following release specifications: 35 to 295×10⁶ cells with >70% cell viability; 5% to 55% of cells were macrophages.

We report here the results of 2 phase 2A clinical trials of intramyocardial delivery of ixmyelocel-T in patients with end-stage HF because of ischemic and nonischemic dilated cardiomyopathy (DCM).
Definitions and End Points
The primary objective of the studies was to assess the safety of ixmyelocel-T. The secondary objectives were to assess the efficacy of ixmyelocel-T when compared with control (standard of care) within each DCM stratum (ischemic and nonischemic) and pooled across strata. Efficacy was evaluated at 1 (IMPACT-DCM only), 3, 6, and 12 months. Major adverse cardiac events (MACE) included cardiac death, cardiac arrest, myocardial infarction, sustained ventricular arrhythmia (eg, ventricular tachycardia or ventricular fibrillation), pulmonary edema, HF exacerbation requiring hospitalization (eg, acute HF), unstable angina, or major bleeding (defined as the need for ≥2 units of blood within 1 week of injection procedure or the need for operation because of bleeding). Changes from baseline in NYHA HF status, Minnesota Living with Heart Failure Questionnaire (MLHFQ), and in exercise tolerance measured by 6-minute walk test were evaluated, as well as C-reactive protein and brain natriuretic peptide. Structural assessments from echocardiogram and SPECT (single-photon emission compute tomography) were read by a blinded core laboratory and included changes from baseline in LVEF, LV dimensions and volumes, wall motion score index, and myocardial perfusion.

Data Analysis
Both studies were phase 2A and designed to evaluate safety and explore potential efficacy. Neither study was powered to test a prospective hypothesis. A computer-generated randomization schedule was used to assign patients within each stratum. Control patients underwent an initial follow-up visit ≈30 days after their screening visit, which was considered day 0 and baseline for data display and summary of adverse events. There was no imputation for missing data. Data from the randomized portion of the study were summarized using descriptive statistics. Differences between groups in the change from baseline were analyzed using a 2 sample unpaired t test. Differences between groups in baseline demographics were compared by an unpaired t test. The proportion of patients who were men, white, experienced a MACE, or achieved a NYHA class I/II was tested using a 2-tailed Fisher exact test. A P value of <0.05 was considered statistically significant. MACEs were adjudicated in a blinded fashion by the Principal Investigators (T.D.H. and A.N.P.). MACE was summarized by the number of patients who experienced a MACE overall and by the number of patients experiencing a specific event categorized as MACE. Treatment-emergent adverse events were summarized by the number of events per patient. For this analysis, a patient was counted only once, regardless of the number of MACE events experienced.

Results
Study Disposition
In the IMPACT-DCM study (n=39), 24 of 25 patients randomized to ixmyelocel-T treatment were treated and 14 patients were in the standard of care (control) group (Figure 1). One aspirate had an inadequate number of mononuclear cells for expansion. In the Catheter-DCM study (n=22), 15 patients were aspirated and received ixmyelocel-T and 7 patients were in the control group. After 6 months in the surgical study or 12 months in the catheter study, 8 control patients met eligibility criteria, underwent a successful bone marrow aspiration, and were subsequently treated with ixmyelocel-T. Between the 2 studies, a total of 61 patients were randomized. A total of 21 patients...
with ischemic DCM received ixmyelocel-T, whereas 9 patients with ischemic DCM served as controls. Eighteen patients with nonischemic DCM received ixmyelocel-T, whereas 11 patients with nonischemic DCM served as controls. A total of 59 patients were evaluated in the combined study results because 1 patient failed aspiration and 1 patient withdrew consent.

Baseline characteristics of the control and ixmyelocel-T–treated populations were similar in both studies (Table 1). In the combined ischemic and nonischemic populations from both studies, the majority of patients were men and white. All but 2 patients were NYHA class III. All ischemic patients were men, whereas nonischemic patients were more likely to be women and slightly younger. Patients with nonischemic cardiomyopathy were a heterogeneous group as expected but did not have coronary artery disease. The Catheter-DCM ischemic control group (n=3) had significantly lower LVEF (15.5%) than the ixmyelocel-T–treated group (n=9; 25.4%). Baseline left ventricular end-diastolic volume, end-systolic volume, 6-minute walk distance (6MWD), and MLHFQ global score values were similar across all other groups. Given the small number of control patients in each strata, data from the 2 studies were combined for end point evaluation.

Table 1. Baseline Characteristics of Patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Characteristic</th>
<th>Ischemic</th>
<th></th>
<th>Nonischemic</th>
<th></th>
<th>Combined</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (n=9)</td>
<td></td>
<td>Ixmyelocel-T (n=21)</td>
<td></td>
<td>P Value</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 (64)</td>
<td>13 (73)</td>
<td>0.69</td>
<td>16 (80)</td>
<td>34 (87)</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.8 (50)</td>
<td>8.12 (67)</td>
<td>0.65</td>
<td>10/14 (71)</td>
<td>20/24 (83)</td>
<td>0.43</td>
</tr>
<tr>
<td>Surgical</td>
<td>Male sex, n (%)</td>
<td>3/3 (100)</td>
<td>9/9 (100)</td>
<td>1.00</td>
<td>3/3 (100)</td>
<td>5/6 (83)</td>
<td>1.00</td>
</tr>
<tr>
<td>Catheter</td>
<td>Age, y, mean (SD)</td>
<td>63.2 (12)</td>
<td>64.7 (9)</td>
<td>0.75</td>
<td>52.3 (11)</td>
<td>57.9 (11)</td>
<td>0.19</td>
</tr>
<tr>
<td>Surgical</td>
<td>Male sex, n (%)</td>
<td>61.3 (13)</td>
<td>63.3 (5)</td>
<td>0.64</td>
<td>54.4 (10)</td>
<td>56.8 (8)</td>
<td>0.56</td>
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<tr>
<td>Catheter</td>
<td>Race, n (%)</td>
<td>67.0 (14)</td>
<td>66.4 (12)</td>
<td>0.94</td>
<td>46.7 (16)</td>
<td>60.3 (16)</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Symptomatic, functional, and echocardiographic measures</td>
<td>10 (91)</td>
<td>15 (83)</td>
<td>1.00</td>
<td>19 (95)</td>
<td>35 (90)</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>NYHA class, n (%)</td>
<td>10 (90)</td>
<td>9 (5)</td>
<td>1.00</td>
<td>13 (73)</td>
<td>17 (94)</td>
<td>1.00</td>
</tr>
<tr>
<td>Combined</td>
<td>LVEF, %, mean (SD)</td>
<td>25.4 (10)</td>
<td>27.2 (7)</td>
<td>0.58</td>
<td>24.7 (6)</td>
<td>25.8 (7)</td>
<td>0.67</td>
</tr>
<tr>
<td>Surgical</td>
<td>LVEDV, mL, mean (SD)</td>
<td>237.3 (42)</td>
<td>204.5 (69)</td>
<td>0.20</td>
<td>223.5 (94)</td>
<td>215.7 (95)</td>
<td>0.83</td>
</tr>
<tr>
<td>Catheter</td>
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<td>215.7 (95)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>6-Min walk distance, m</td>
<td>178.3 (45)</td>
<td>151.6 (50)</td>
<td>0.24</td>
<td>169.0 (72)</td>
<td>163.4 (83)</td>
<td>0.85</td>
</tr>
<tr>
<td>Combined</td>
<td>MLHFQ Global Score, mean (SD)</td>
<td>166.3 (38)</td>
<td>149.2 (74)</td>
<td>0.61</td>
<td>167.5 (72)</td>
<td>171.7 (96)</td>
<td>0.90</td>
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<tr>
<td>Surgical</td>
<td>6-Min walk distance, m</td>
<td>208.5 (60)</td>
<td>154.2 (46)</td>
<td>0.13</td>
<td>172.4 (87)</td>
<td>149.6 (60)</td>
<td>0.65</td>
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<tr>
<td>Catheter</td>
<td>MLHFQ Global Score, mean (SD)</td>
<td>386.3 (33)</td>
<td>357.7 (81)</td>
<td>0.32</td>
<td>375.5 (64)</td>
<td>379.1 (97)</td>
<td>0.97</td>
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<td>Surgical</td>
<td>6-Min walk distance, m</td>
<td>374.8 (14)</td>
<td>368.9 (69)</td>
<td>0.84</td>
<td>368.3 (58)</td>
<td>369.0 (105)</td>
<td>0.99</td>
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<tr>
<td>Catheter</td>
<td>MLHFQ Global Score, mean (SD)</td>
<td>409.3 (53)</td>
<td>342.8 (98)</td>
<td>0.30</td>
<td>395.0 (91)</td>
<td>399.3 (84)</td>
<td>0.95</td>
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<tr>
<td>Surgical</td>
<td>6-Min walk distance, m</td>
<td>54.9 (28)</td>
<td>46.5 (23)</td>
<td>0.40</td>
<td>48.4 (22)</td>
<td>55.8 (21)</td>
<td>0.37</td>
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<tr>
<td>Catheter</td>
<td>MLHFQ Global Score, mean (SD)</td>
<td>47.0 (28)</td>
<td>49.7 (24)</td>
<td>0.83</td>
<td>51.1 (21)</td>
<td>53.9 (21)</td>
<td>0.77</td>
</tr>
<tr>
<td>Surgical</td>
<td>6-Min walk distance, m</td>
<td>70.7 (24)</td>
<td>42.3 (23)</td>
<td>0.10</td>
<td>41.0 (26)</td>
<td>59.7 (21)</td>
<td>0.28</td>
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<td>0.83</td>
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</tr>
</tbody>
</table>

LVEDV indicates left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; NYHA, New York Heart Association; and MLHFQ, Minnesota Living with Heart Failure Questionnaire.
Table 2. Summary of Adverse Events Per Patient

<table>
<thead>
<tr>
<th>Days 0–5</th>
<th>Days 6–730</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical study (IMPACT-DCM)</td>
<td>6.71</td>
</tr>
<tr>
<td>Catheter study (Catheter-DCM)</td>
<td>0.93</td>
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</table>

DCM indicates dilated cardiomyopathy.

finding was not observed in the catheter study. After the injection procedure, the number of AEs per patient in both groups was comparable in both studies.

In the IMPACT-DCM surgical study, 2 of 24 (8%) ixmyelocel-T–treated and 1 of 14 (7%) control patients died during the 6-month follow-up period. All 3 patients had ischemic DCM; 1 of the 3 deaths was from a noncardiovascular cause. In the Catheter-DCM study, no patients died during the 1-year follow-up period. There were no heart transplants during the 6-month or 1-year follow-up period. Left ventricular assist device placements occurred in 1 of 14 (7%) control and 3 of 24 (12.5%) nonischemic-treated patients during the 6-month or 1-year follow-up period.

Efficacy

Fewer ischemic patients treated with ixmyelocel-T experienced a MACE after injection when compared with control (Table 3). A similar benefit was not seen in the nonischemic patients. HF exacerbation was the most common MACE (Table 4). Both ventricular arrhythmia events occurred in the surgical study during surgery. Treatment with ixmyelocel-T was associated with a significant improvement with NYHA class and 6MWD, as well as a trend in MLHFQ scores in the ischemic population relative to control (Figure 2). Differences in NYHA class between treatment groups were statistically significant as early as 1 month and 6MWD reached statistical significance at 12 months. Of the patients treated with ixmyelocel-T, there was not a statistically significant difference in these end points between the ischemic and the nonischemic groups. Both physical and emotional domain scores of the MLHFQ showed improvement in the ischemic ixmyelocel-T–treated group. Similar trends were observed for both studies individually.

There was no difference in the change from baseline in LVEF, left ventricular end-diastolic volume, and left ventricular end-systolic volume in treated patients relative to control in either stratum (Figure 3). There was a trend toward improved wall motion score index in ixmyelocel-T–treated ischemic patients (Figure 4). This trend was observed in both studies individually as well. Twelve months after treatment, there was no change from baseline in stroke volume and cardiac output in any treatment group. No differences from baseline were observed in C-reactive protein (mean±SEM) at 3 months (4.7±0.9 versus 5.8±1.4 mg/L) or brain natriuretic peptide (mean±SEM) at 12 months (502±71 versus 451±63 ng/L) in the ixmyelocel-T–treated patients.

**Discussion**

The objective of these 2 studies was to evaluate the safety and feasibility of 2 methods of ixmyelocel-T cell delivery, as well as to identify potential clinical benefit. Despite a small number of patients treated in the individual studies, the combined data suggest that intramyocardial injection with ixmyelocel-T reduces MACE and improves symptoms in patients with ischemic DCM but not in patients with nonischemic DCM. Given the similar study design, including stratification by ischemic versus nonischemic cause, similar eligibility criteria, and patient follow-up, we elected to present both studies together. There were other slight differences between the studies, including the pattern of intramyocardial injection pattern.

On the basis of the increased incidence of adverse events associated with ixmyelocel-T administration via minimally invasive thoracoscopy or lateral thoracotomy compared with the catheter administration, we selected catheter administration for an ongoing phase 2 double-blind, placebo-controlled trial in patients with ischemic cardiomyopathy (ClinicalTrials.gov Identifier: NCT01670981). Notably, the number of SAEs in treated or standard of care patients in both studies did not differ during the 2-year follow-up period, starting on day 6. Numeric and clinically meaningful improvement in NYHA class, MLHFQ score, and 6MWD was observed in the ischemic patients starting 1 month after treatment and was sustained through 12 months. In addition, the number of MACE was lower in the treated ischemic patients when compared with that in the ischemic control patients, even when considering that 2 of the MACE (ventricular arrhythmia) in the treated patients seem to be related to the surgical procedure. The improvement in LV function from cell therapy overall has been moderate. Although the cell types are similar to those found in the BMMC population, the numbers of CD90+ and CD14+Auto+ cells are significantly greater in ixmyelocel-T. The prevailing scientific view is that a mixture of regenerative cell types, such as mesenchymal stromal cells and alternatively activated macrophages (CD90+ and CD14+Auto+, respectively), rather than a single cell type, are required to promote long-term tissue regeneration and repair.22,23 On the basis of preclinical data, we found that ixmyelocel-T provides benefit via a
multimodal mechanism of action, including a local paracrine effect given its cytokine expression profile. Either the CD90+ or CD14+Auto+ cells from ixmyelocel-T secrete 10-fold more anti-inflammatory cytokines interleukin 1-RA, interleukin-10, macrophage inflammatory protein-1α and growth factors vascular endothelial growth factor and hepatocyte growth factor than BMMCs. In a rat model of chronic arterial occlusion, nonclassically activated anti-inflammatory macrophages (such as the CD14+Auto+ macrophages in ixmyelocel-T) have been demonstrated to play a role during collateral growth. M2 macrophages increased in number in the perivascular space after occlusion. Interleukin-10 treatment, known to induce M2 activation, led to perfusion recovery, indicating that the M2 macrophage is critical for collateral growth.

Limitations

The major limitation for both trials is the lack of true placebo groups. A recent meta-analysis of cell therapy treatment after an acute myocardial infarction suggests that the lack of placebo may overestimate the treatment effect. Either the CD90+ or CD14+Auto+ cells from ixmyelocel-T secrete 10-fold more anti-inflammatory cytokines interleukin 1-ra, interleukin-10, macrophage inflammatory protein-1α and growth factors vascular endothelial growth factor and hepatocyte growth factor than BMMCs. In a rat model of chronic arterial occlusion, nonclassically activated anti-inflammatory macrophages (such as the CD14+Auto+ macrophages in ixmyelocel-T) have been demonstrated to play a role during collateral growth. M2 macrophages increased in number in the perivascular space after occlusion. Interleukin-10 treatment, known to induce M2 activation, led to perfusion recovery, indicating that the M2 macrophage is critical for collateral growth.

Limitations

The major limitation for both trials is the lack of true placebo groups. A recent meta-analysis of cell therapy treatment after an acute myocardial infarction suggests that the lack of placebo may overestimate the treatment effect. We noted differences in both the standard of care populations and treated patients based on the cause of DCM, with higher events in the ischemia patients. Assuming the placebo effect on secondary end points, such as NYHA class, 6MWD, and MLHFQ, would be the same in the 2 treated populations; these results

Table 4. Listing of MACE by Strata and Treatment Group

<table>
<thead>
<tr>
<th>MACE No./N (%) of Patients</th>
<th>Ischemic</th>
<th>Nonischemic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Cardiac death</td>
<td>1 (11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Ventricular arrhythmia†</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>CHF exacerbation</td>
<td>4 (44)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Major bleeding</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

CHF indicates congestive heart failure; and MACE, major adverse cardiac event.

*Patients may have had multiple events in different MACE categories; therefore, columns with specific events do not add up to the row value for any MACE shown in Table 3.

†Two cases of ventricular arrhythmia occurred during the surgical procedure.
suggest that there is a greater treatment effect in the ischemic population. When compared with the nonischemic ixmyelocel-T–treated patients, the ischemic-treated patients had a greater improvement in NYHA class, 6MWD, and MLHFQ scores. When compared with the ischemic control population, control patients in the nonischemic population tended to show improvement in NYHA class and 6MWD during the 12 months of assessments, potentially diminishing the ability to detect a beneficial treatment effect of ixmyelocel-T. However, the significant improvement of NYHA class and 6MWD in the ischemic patients from the ixmyelocel-T group is in contrast with the lack of changes of cardiac structure and function and may be partially explained by the placebo effect in the absence of binding and sham-treated control groups. This is currently being tested in an ongoing phase 2 double-blind, placebo-controlled trial, ixCELL-DCM.

Conclusions
In summary, catheter administration of ixmyelocel-T has a superior safety profile when compared with surgical administration. The clinical benefit of ixmyelocel-T was more pronounced in patients with ischemic DCM. These results provide the rationale to evaluate the catheter delivery of ixmyelocel-T for the treatment of ischemic DCM in a double-blind placebo-controlled study.

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References
11. Hare JM, Fishman JE, Gerstenblith G, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by...


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**Novelty and Significance**

**What Is Known?**

- Results of clinical trials with bone marrow mononuclear cells demonstrate safety but only modest efficacy.
- The number and potency of stem cells decline with age and cardiac risk factors.
- Ixmyelocel-T is an expanded multicellular therapy cultured from autologous bone marrow mononuclear cells that contain ≥200- and 50-fold the number of M2-like macrophages and mesenchymal stem cells than bone marrow mononuclear cells, respectively.

**What New Information Does This Article Contribute?**

- In 2 randomized, open-label studies, intramyocardial injection with ixmyelocel-T was associated with a reduction in the number of patients with major adverse cardiovascular events and improved symptoms in patients with ischemic dilated cardiomyopathy (DCM) but not in patients with nonischemic DCM.
- There were fewer adverse events associated with ixmyelocel-T administration via catheter in comparison with minimally invasive thoracoscopy or lateral thoracotomy.
- These results strengthen the rationale for the design of the ongoing phase 2B randomized double-blind, placebo-controlled ixCELL-DCM trial.

Administration of autologous bone marrow mononuclear cells for the treatment of heart failure has been shown to be well tolerated, but efficacy is limited. As a potential improvement to the treatment with autologous bone marrow mononuclear cells, ixmyelocel-T, an expanded and enhanced stem cell therapy agent was evaluated for the treatment of ischemic and nonischemic DCM. Two randomized, open-label studies were conducted to evaluate the safety and feasibility of 2 methods of ixmyelocel-T cell delivery, as well as to identify potential clinical benefit. Improvement in New York Heart Association class, Minnesota Living with Heart Failure Questionnaire score, and 6-minute walk distance was observed in the ischemic patients starting 1 month after the treatment and sustained through 12 months. In addition, the number of major adverse cardiac events was lower in the patients with ischemic-treated DCM when compared with patients with ischemic control DCM. Similar improvements were not observed in the nonischemic DCM population. In comparison with minimally invasive thoracoscopy or lateral thoracotomy, there were fewer adverse events associated with percutaneous intramyocardial ixmyelocel-T administration. The results of these studies provide the groundwork for the ongoing phase 2B randomized double-blind, placebo-controlled trial—ixCell-DCM, involving intramyocardial delivery of ixmyelocel-T to ischemic DCM patients.
Safety and Efficacy of Ixmyelocel-T: An Expanded, Autologous Multi-Cellular Therapy, in Dilated Cardiomyopathy
Timothy D. Henry, Jay H. Traverse, Baron L. Hammon, Cara A. East, Brian Bruckner, Ann E. Remmers, David Recker, David A. Bull and Amit N. Patel

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Safety and Efficacy of Ixmyelocel-T: An Expanded, Autologous Multi-Cellular Therapy, in Dilated Cardiomyopathy

Supplemental Material

Supplemental Table

<table>
<thead>
<tr>
<th>Summary of Deaths, LVAD placements, and Heart Transplants</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMPACT-DCM (6 months)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Number/N (%) of Deaths Ischemic</td>
</tr>
<tr>
<td>Nonischemic</td>
</tr>
<tr>
<td>Number/N (%) of Heart Transplants Ischemic</td>
</tr>
<tr>
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</tr>
<tr>
<td>Number/N (%) of LVAD placements Ischemic</td>
</tr>
<tr>
<td>Nonischemic</td>
</tr>
</tbody>
</table>

Supplemental Methods

Impact-DCM surgical study eligibility criteria

Inclusion Criteria

1. Diagnosis of ischemic or nonischemic dilated cardiomyopathy according to WHO criteria:
   - Dilatation and impaired contraction of the left ventricle or both ventricles of idiopathic, familial/genetic, viral and/or immune, toxic origin, or associated with recognized cardiovascular disease in which the degree of myocardial dysfunction is not explained by the abnormal loading conditions or the extent of ischemic damage (Richardson P; et al., 1996)
   
   **Or**
   - Ischemic dilated cardiomyopathy is defined as dilated cardiomyopathy in a patient with a history of myocardial infarction or evidence of clinically significant (≥70% narrowing of a major epicardial artery) coronary artery disease (Bristow MR; et al., 1991).

2. No other cardiac surgery or percutaneous cardiac interventions are likely to produce clinical improvement, in the opinion of the investigator (cardiac surgeon) and the referring interventional cardiologist.

3. Left ventricular ejection fraction ≤30% by echocardiogram.

4. Symptomatic heart failure in NYHA functional class III or IV defined by:
- NYHA class III: Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.

- NYHA class IV: Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased. Note: Only those patients who can tolerate surgery and are not actively receiving inotropes should be included.

5. Able to comply with scheduled visits in cardiac out-patient clinic.

6. Able to tolerate study procedures, including bone marrow aspiration, left lateral thoracotomy or thoracoscopy with single lung ventilation, MRI or cardiac CT, spirometry and 6 minute walk test.

7. Males and females, 18-86 years of age.

8. Life expectancy of 6 months or more in the opinion of the investigator.

9. Able to give informed consent.

10. Normal organ and marrow function as defined:
    - Leukocytes $\geq 3,000/\mu L$
    - Absolute neutrophil count $\geq 1,500/\mu L$
    - Platelets $\geq 140,000/\mu L$
    - AST (SGOT)/ALT (SGPT) $\leq 2.5 \times$ institutional standards range
    - Creatinine $\leq 2.5 \text{ mg/dL}$

11. Adequate pulmonary function defined by forced expiratory volume in one second (FEV1) $>50\%$ of predicted.

12. Controlled blood pressure (defined as a systolic blood pressure $\leq 140$ and a diastolic blood pressure of $\leq 90 \text{ mmHg}$) and established anti-hypertensive therapy as necessary prior to entry into the study.

13. Adequate medical management of DCM and other pre-existing conditions (e.g., hypercholesterolemia, thromboembolic risk).

    The adequate medical management of DCM includes the following:
    - Placement of an automated implantable cardioversion defibrillator (AICD) unless contraindicated (e.g., due to patient profession). Patient refusal of AICD placement is not considered a valid contraindication.
    - Use of ACE inhibitors and/or AT-1 receptor blockers as well as loop diuretics (e.g., furosemide).
    - Depending on the type of heart failure associated with the disease, therapy may also include the use of vasodilators (e.g., nitrates or hydralazine), beta blockers (e.g. long-acting metprolol or carvedilol), digoxin, and aldosterone antagonists (e.g. spironolactone or canrenoate).
A drug treatment regimen for their DCM must have been established for at least one month with no new medications to treat the disease introduced in the last 3 months.

14. Fertile patients must agree to use an appropriate form of contraception while participating in the study.

**Exclusion Criteria**

1. Severe primary valvular heart disease including, but not limited to, aortic valve stenosis and insufficiency.

2. Known history of Chronic Obstructive Pulmonary Disease (COPD) defined as Gold stages IIB (FEV1/FVC<70% with FEV1<50% predicted, with or without chronic symptoms of cough, sputum production, dyspnea) or more severe or restrictive pulmonary disease.

3. Known history of primary pulmonary hypertension.

4. Ventricular Assist Device (VAD) implantation.

5. Myocardial infarction within 4 weeks prior to randomization.

6. Life-threatening ventricular arrhythmia, except if an implantable cardioverter defibrillator (ICD) is implanted.

7. Unstable angina, characterized by increasingly frequent episodes with modest exertion or at rest, worsening severity, and prolonged duration.

8. Patients receiving treatment with hematopoietic growth factors (e.g. EPO, GM-CSF).

9. Patients who require uninterruptible anticoagulation or anti-platelet therapy [i.e. anticoagulation therapy (e.g. warfarin) that cannot be stopped for 72 hours prior to bone marrow aspiration and intramyocardial injections].

**And**

Patients receiving anti-platelet therapy (e.g. clopidogrel) that cannot be stopped for 7 days prior to bone marrow aspiration and intramyocardial injections.

10. Known cancer and undergoing treatment including chemotherapy and radiotherapy.

11. Patients who will require continuous, systemic, high dose corticosteroid therapy (more than 7.5 mg/day) within 6 months after surgery.

12. End stage renal disease requiring dialysis.

13. Patients who are pregnant or lactating; positive for hCG.

14. History of alcohol consumption regularly exceeding the equivalent of 2 drinks/day (1 drink = 5 oz of wine or 12 oz [360mL] of beer or 1.5 oz [45mL] of hard liquor or history of illicit drug use within 6 months of screening.

15. Known allergies to protein products (horse or bovine serum, or porcine trypsin) used in the ex-vivo cell production process.

16. Body Mass Index (BMI) of 40 Kg/m² or greater.
17. Patients receiving experimental medications or participating in another clinical study within 30 days of screening.
18. HIV or syphilis, positive at time of screening.
19. Active Hepatitis B, or Hepatitis C infection at the time of screening.
20. In the opinion of the investigator, the patient is unsuitable for cellular therapy.
21. Patients receiving anti-angiogenic drugs (e.g. anti-VEGF).

Catheter-DCM study eligibility criteria

Inclusion Criteria

1. Diagnosis of ischemic or non-ischemic dilated cardiomyopathy according to WHO criteria:
   - Ischemic dilated cardiomyopathy is defined as dilated cardiomyopathy in a patient with a history of myocardial infarction or evidence of clinically significant ($\geq 70\%$ narrowing of a major epicardial artery) coronary artery disease (Bristow MR; et al., 1991).
   - Non-ischemic dilated cardiomyopathy is defined as dilatation and impaired contraction of the left ventricle or both ventricles of idiopathic, familial/genetic, viral and/or immune, toxic origin, or associated with recognized cardiovascular disease in which the degree of myocardial dysfunction is not explained by the abnormal loading conditions or the extent of ischemic damage (Richardson P; et al., 1996).

2. No other cardiac surgery or percutaneous cardiac interventions are likely to produce clinical improvement and confirmed by an interventional cardiologist (for PTCA) and a cardiothoracic surgeon (for CABG). This condition is satisfied in patients with chronic ischemic disease when a patient has previously been successfully revascularized but has failed to show clinical improvement. All patients who are candidates for revascularization are considered not eligible for participation in the study. (For patients diagnosed with non-ischemic disease, there is no need for a cardiothoracic surgeon consult.)

3. Left ventricular ejection fraction $\leq 30\%$ by echocardiogram, per assessment performed within 30 days prior to randomization.

4. Symptomatic heart failure in NYHA functional class III or IV defined by:
   - NYHA class III: Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
   - NYHA class IV: Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

5. Able to comply with scheduled visits in cardiac out-patient clinic.
6. Able to tolerate study procedures, including bone marrow aspiration, metabolic stress test, 6 minute walk test. Patients must also be able to tolerate NOGA mapping.

7. Males and females, 18-86 years of age.

8. Life expectancy of 6 months or more in the opinion of the investigator.

9. Able to give informed consent.

10. Normal organ and marrow function as defined:
    - Leukocytes $\geq 3,000/\mu$L
    - Absolute neutrophil count $\geq 1,500/\mu$L
    - Platelets $\geq 140,000/\mu$L
    - AST (SGOT)/ALT (SGPT) $\leq 2.5 \times$ institutional standards range
    - Creatinine $\leq 2.5$ mg/dL

11. Controlled blood pressure (defined as a systolic blood pressure $\leq 140$ and a diastolic blood pressure of $\leq 90$ mmHg) and established anti-hypertensive therapy as necessary prior to entry into the study.

12. Patient has received stable, standard medical therapy for DCM for at least one month with no new medications to treat the disease introduced in the last 3 months. Standard medical therapy includes the following:
    - Placement of an automated implantable cardioverter defibrillator (AICD) unless contraindicated (e.g., due to patient profession, etc.). Patient refusal of AICD placement is not considered a valid contraindication. If a bi-ventricular pacer/ICD has been placed, the patient must wait 3 months from time of placement before randomization.
    - Use of ACE inhibitors and/or AT-1 receptor blockers unless contraindicated, and use of loop diuretics (e.g., furosemide) as dictated by a patient’s current medical condition.
    - Depending on the type of heart failure associated with the disease, standard therapy may also include the use of vasodilators (e.g., nitrates or hydralazine), beta blockers (e.g. long-acting metoprolol or carvedilol), digoxin, and aldosterone antagonists (e.g. spironolactone or canrenoate), or other medications.

13. Pre-existing conditions (e.g., hypercholesterolemia, thromboembolic risk, diabetes) are adequately controlled in the opinion of the investigator.

14. Fertile patients (male and female) must agree to use an appropriate form of contraception while participating in the study.

**Exclusion Criteria**

1. Severe primary valvular heart disease including, but not limited to, aortic valve stenosis and insufficiency. Patients with aortic valve prosthesis, artificial or animal derived, are also excluded.
2. Known history of Chronic Obstructive Pulmonary Disease (COPD) defined as Gold stage IIB (FEV1/FVC<70% with FEV1 30% - 49% of predicted, with or without chronic symptoms of cough, sputum production, dyspnea) or more severe or restrictive pulmonary disease.
3. Known history of primary pulmonary hypertension.
4. Ventricular Assist Device (VAD) implantation.
5. Myocardial infarction within 4 weeks prior to randomization.
6. History of life-threatening ventricular arrhythmia, except if an automated implantable cardioverter defibrillator (AICD) is implanted.
7. Unstable angina, characterized by increasingly frequent episodes with modest exertion or at rest, worsening severity, and prolonged duration.
8. Patients who are at high risk for complications due to the injection procedure (e.g., patients who have severe peripheral atherosclerotic disease that does not allow advancement of the catheter; patients who have a prosthetic aortic or mitral valve; patients who have a left ventricular thrombus or aneurysm; patients who have an aortic dissection or aneurysm, etc.).
9. Patients with poorly controlled diabetes mellitus (HbA1c > 9.0%).
10. Patients receiving treatment with hematopoietic growth factors (e.g., EPO, G-CSF).
11. Patients who are unable to tolerate institutional guidelines regarding anticoagulant and anti-platelet therapy during bone marrow aspiration and transendocardial injections.
12. Known cancer and undergoing treatment including chemotherapy and radiotherapy.
13. Patients who will require continuous, systemic, high dose corticosteroid therapy (more than 7.5 mg/day) within 1 month before aspiration or 6 months after injection procedure.
14. End stage renal disease requiring dialysis.
15. Patients who are pregnant or lactating; positive for hCG
16. History of alcohol consumption regularly exceeding the equivalent of 2 drinks/day (1 drink = 5 oz of wine or 12 oz [360mL] of beer or 1.5 oz [45mL] of hard liquor or history of illicit drug use within 6 months of screening.
17. Known allergies to protein products (horse or bovine serum, or porcine trypsin) used in the ex-vivo cell production process.
18. Body Mass Index (BMI) of 40 Kg/m² or greater.
19. Patients receiving experimental medications or participating in another clinical study within 30 days of screening.
20. HIV or syphilis, positive at time of screening.
21. Active Hepatitis B or Hepatitis C infection at the time of screening.
22. In the opinion of the investigator or the sponsor, the patient is unsuitable for cellular therapy.

23. Patients receiving anti-angiogenic drugs (e.g. anti-VEGF).

24. In the opinion of the investigator, the patient’s LV wall thickness is unsuitable for cell injections.

**Supplemental References**
