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

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ABSTRACT

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

Objectives: To evaluate the safety and efficacy of ixmyelocel-T in 2 prospective randomized Phase 2A Trials administered via mini-thoracotomy or intramyocardial catheter injections in patients with dilated cardiomyopathy (DCM) stratified by ischemic or non-ischemic 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) and ixmyelocel-T was administered intramyocardially via mini-thoracotomy. 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 two 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 (MACE) during follow up compared to control patients. A similar benefit was not seen in the non-ischemic patients. Heart failure (HF) exacerbation was the most common MACE. Ixmyelocel-T treatment was associated with improved NYHA 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 non-ischemic population.

Conclusions: Intramyocardial injection with ixmyelocel-T reduces MACE and improves symptoms in patients with ischemic DCM but not non-ischemic DCM.

Keywords: Heart Failure, stem cell therapy, dilated cardiomyopathy, clinical trial, autologous

Nonstandard Abbreviations Acronyms:

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<th>Description</th>
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<tr>
<td>6MWD</td>
<td>6-minute Walk Distance based on 6-minute walk test</td>
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<tr>
<td>BMMC</td>
<td>Bone marrow mononuclear cells</td>
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<td>BNP</td>
<td>Brain natriuretic peptide</td>
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<td>LVEF</td>
<td>Left Ventricle Ejection Fraction</td>
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<td>LVAD</td>
<td>Left Ventricular Assist Device</td>
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<td>MACE</td>
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<td>MLHFQ</td>
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INTRODUCTION

Heart failure (HF) remains a major public health burden, affecting approximately 5.1 million adults in the United States (US). Approximately 50% of patients diagnosed with HF will die within five years and the prevalence is growing. Improvements in the pharmacologic and surgical management of patients with cardiovascular disease has 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/or 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 stem 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 use of allogeneic cells (11), cardiac-derived cells (12, 13), selected cell populations such as CD34+ cells(14), and expanded or enhanced autologous BMMC (15, 16).

Ixmyelocel-T is an expanded multi-cellular therapy cultured from autologous BMMC comprised of myeloid cells (i.e., granulocytes, monocytes, and mixed myeloid progenitors) and lymphoid cell types (i.e., T-cells, B-cells and mixed lymphoid progenitors) that express CD45+ on the cell surface, as well as CD90+ mesenchymal stromal cells (MSCs) (16). Within the population of CD45+ cells is a subpopulation of CD45+CD14+ auto-fluorescent (CD14+ Auto+) M2-like macrophages. While 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 to the relatively small reservoir of these two cell types in bone marrow, ixmyelocel-T contains approximately 200-fold and 50-fold the number of M2 macrophages and MSCs, respectively. A range of biological activities relevant to tissue repair and regeneration have been demonstrated reflecting the multicellular composition of ixmyelocel-T (16-18). Additionally, ixmyelocel-T was associated with improved ischemic ulcer healing and a reduction in the rate of amputation in a placebo-controlled phase 2 study of in patients with critical limb ischemia (19).

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

METHODS

Study design.

Two prospective, randomized, open-label, multi-center, phase 2Atrials were conducted to assess the safety and efficacy of ixmyelocel-T administered via mini-thoracotomy or intramyocardial catheter injections with the NOGA® Myostar in patients with DCM stratified by ischemic or non-ischemic status. The surgical study (IMPACT-DCM; ClinicalTrials.gov Identifier: NCT00765518) was conducted from November 2008 through September 2012. Eligible patients were randomized to either ixmyelocel-T or standard of care control 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 control in a 2:1 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 FDA. 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.

**Patient selection.**
Both studies enrolled a high-risk patient population with ischemic or non-ischemic DCM based on the following criteria: [World Health Organization Definitions and Classifications (20)], symptomatic HF, New York Heart Association (NYHA) Class III or IV, left ventricular ejection fraction (LVEF) ≤30%, and ineligibility for percutaneous or surgical revascularization.

Patients with ischemic dilated cardiomyopathy had a history of myocardial infarction (MI) or evidence of clinically significant (≥ 70% narrowing of a major epicardial artery) coronary artery disease. Patients without a history of MI were required to have coronary angiography within the past 5 years. As part of the prescreening process, either the Aastrom medical monitor and/or Principle Investigators (TDH and ANP) reviewed available medical history jointly with the Investigator to evaluate if a patient could be screened. Eligibility for revascularization was determined by the local investigator 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, uncontrolled atrial fibrillation) were also used in Catheter-DCM. (21).

**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 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+ autofluorescent (CD14+Auto+) macrophages. All products met the following release specifications: 35 to 295 x 10⁶ cells with >70% cell viability; 5 to 55% of cells were CD90+, and 45 to 95% of cells were CD45+. Following the cell expansion process, ixmyelocel-T was returned to the clinical site in a labeled patient-specific bag for injection administration.

In IMPACT-DCM, patients received ixmyelocel-T injections via minimally invasive thoracotomy or lateral thoracotomy (determined by the treating cardiac surgeon) to approximately 25-30 injection sites equally distributed (medially and laterally) across the anterior and posterior areas of the left ventricular wall. Injection sites were a minimum of 1 cm apart, and designed to encompass as much of the ventricular-free wall as possible. Each injection contained 0.2 mL of ixmyelocel-T suspension delivered via a 1 mL syringe into the mid-myocardium at each of the injection sites.

In Catheter-DCM, ixmyelocel-T was delivered percutaneously via the NOGA® XP cardiac navigation system (Biologics Delivery Systems Group of Cordis Corporation, a Johnson & Johnson company; Irwindale, CA) (14) with a series of 12-20 injections of 0.4 mL each at least 1 cm apart into the myocardium.
For ischemic patients, ixmyelocel-T injections were performed within 2 cm of the border between viable and infarcted (<6 mV unipolar voltage) myocardium. For nonischemic patients, ixmyelocel-T injections were distributed equally among the anterior, lateral, and posterior left ventricular wall, avoiding the intraventricular septum and areas where wall thickness was less than 6 mm. All injections were made into viable myocardium with unipolar voltage ≥6.0 mV and loop stability <3 mm. The mean (+-SD) number of injections for Catheter-DCM was 13+- 1.6 and for Impact-DCM 25 +/- 2.6.

Patients in the control group received standard-of-care treatment for DCM, according to accepted medical practices. Following a data review by the Data Safety Monitoring Board, patients in the control group were allowed to be re-screened for an extension study to receive ixmyelocel-T after 6-months of follow-up in IMPACT-DCM or 12 months of follow-up in Catheter-DCM.

Definitions and endpoints.
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 compared with control (standard of care) within each DCM stratum (ischemic and non-ischemic) 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, MI, sustained ventricular arrhythmia (e.g. ventricular tachycardia or ventricular fibrillation), pulmonary edema, HF exacerbation requiring hospitalization (e.g. acute HF), unstable angina, or major bleeding (defined as the need for 2 or more units of blood within 1 week of injection procedure or the need for operation due to bleeding). Changes from baseline in NYHA heart failure 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 (CRP) and brain natriuretic peptide (BNP). Structural assessments from echocardiogram and SPECT were read by a blinded core lab 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 approximately 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 male, white, experienced a MACE, or achieved a NYHA Class I/II was tested using a two-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 (TDH and ANP). 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

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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 two studies, a total of 61 patients were randomized. A total of 21 ischemic DCM patients received ixmyelocel-T while 9 ischemic DCM patients served as controls. Eighteen non-ischemic DCM patients received ixmyelocel-T while 11 non-ischemic DCM patients served as controls. A total of 39 patients were evaluated in the combined study results since 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 non-ischemic populations from both studies, the majority of patients were male and white. All but two patients were NYHA Class III. All ischemic patients were men, while nonischemic patients were more likely to be women and slightly younger. Patients with non-ischemic 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, and MLHFQ global score values were similar across all other groups. Given the small number of control patients in each strata, data from the two studies was combined for endpoint evaluation.

Safety.

The mean number of adverse events per patient is shown for individual studies by treatment group and displayed by Days 0 to 5 and Day 6 through 730 (Table 2). Only ixmyelocel-T-treated patients underwent mini-thoracotomy, thoroscopy, or catheter procedure. The five most common adverse events that occurred in the ixmyelocel-T group (ischemic and nonischemic) during the surgical procedure (Days 0-5) were hypotension, nausea, constipation, hyperglycemia, and hypertension. Surgical delivery of ixmyelocel-T was associated with a higher incidence of SAEs in the peri-surgical period (Days 0 through 5). A similar finding was not observed in the catheter study. Following 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 were ischemic DCM patients and one was a non-cardiovascular death (supplemental table). 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 (LVAD) placements occurred in 1/14 (7%) control and 3/24 (12.5%) treated non-ischemic patients during the 6 month or 1 year follow-up period.

Efficacy.

Fewer ischemic patients treated with ixmyelocel-T experienced a MACE following injection compared to control (Table 3). A similar benefit was not seen in the non-ischemic 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 6-minute walk distance as well as a trend in MLHFQ scores in the ischemic population relative to control (Figure 2). Differences in NYHA Class between treatment groups was statistically significant as early as 1 month and 6-minute walk distance reached statistical significance at 12 months. Of the patients treated with ixmyelocel-T, there was not a statistically significant difference in these endpoints between the ischemic and 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 post-treatment, there was no change from baseline in stroke volume and cardiac output in any treatment group. No differences from baseline were observed in CRP (mean ± SEM) at 3 months (4.7 ± 0.9 mg/L vs 5.8 ± 1.4 mg/L) or BNP (mean ± SEM) at 12 months (502 ± 71 ng/L vs 451 ± 63 ng/L) in the ixmyelocel-T treated patients.

DISCUSSION

The objective of these two studies was to evaluate the safety and feasibility of two 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 dilated cardiomyopathy (DCM) but not non-ischemic DCM. Given the similar study design, including stratification by ischemic versus non-ischemic etiology, 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.

Based on the increased incidence of adverse events associated with ixmyelocel-T administration via minimally invasive thoracoscopy or lateral thoracotomy compared to the catheter administration, catheter administration was selected 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. Numerical and clinically meaningful improvement in NYHA Class, MLHFQ score, and 6-minute walk distance 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 compared to ischemic control patients, even when considering that two of the MACE (ventricular arrhythmia) in the treated patients appear to be related to the surgical procedure. The improvement in LV function from cell therapy overall has been moderate (5-7).

While 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 like MSCs 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]. Based on preclinical data, ixmyelocel-T provides benefit via a multimodal mechanism of action, including a local paracrine effect given its cytokine expression profile (16). Either the CD90+ or CD14+Auto+ cells from ixmyelocel-T secrete 10-fold more anti-inflammatory cytokines IL1-ra, IL-10, MIP-1α and growth factors vascular endothelial growth factor and hepatocyte growth factor than BMMCs. In a rat model of chronic arterial occlusion, non-classically activated anti-inflammatory macrophages (such as the CD14+Auto macrophages in ixmyelocel-T) have been demonstrated to play a role during collateral growth (24). M2 macrophages increased in number in the perivascular space following occlusion. IL-10 treatment, known to induce M2 activation, led to perfusion recovery, indicating that the M2 macrophage is critical for collateral growth.

Study limitations.

The major limitation for both trials is the lack of true placebo groups. A recent meta-analysis of cell therapy treatment following an acute myocardial infarction suggests that lack of placebo may overestimate the treatment effect (25). We noted differences in both the standard of care populations and treated patients based on the etiology of DCM, with higher events in the ischemia patients. Assuming the
“placebo effect” on secondary endpoints such as NYHA Class, 6-minute walk distance and MLHFQ would be the same in the two treated populations; these results suggest there is a greater treatment effect in the ischemic population. Compared to the non-ischemic ixmyelocel-T-treated patients, the ischemic treated patients had a greater improvement in NYHA class, 6-minute walk distance, and MLHFQ scores. Compared to the ischemic control population, control patients in the non-ischemic population tended to show improvement in NYHA class and 6-minute walk distance over the 12 months of assessments, potentially diminishing the ability to detect a beneficial treatment effect of ixmyelocel-T. None the less, the significant improvement of NYHA class, and 6-minute walk distance 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 blinding and sham-treated control groups. This is currently being tested in an ongoing Phase 2 double-blind, placebo-controlled trial, IxCELL-DCM.

Conclusion

In summary, catheter administration of ixmyelocel-T has a superior safety profile compared to surgical administration. The clinical benefit of ixmyelocel-T was more pronounced in ischemic DCM patients. 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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PARTICIPATORY SITES

Impact-DCM: Clinical Research Coordinators (CRCs) Patty Meldrum and Kristin Kolsch from the University of Utah; CRC Pouak Moshayedi and Patricia Melly PA from Baylor Soltero CV Research, CRC Raquel Bunge from Methodist DeBakey Health Center, Dr Omar Lattouf and CRCs Shannon Smith and Alexis Neill from Emory University, and Dr Nicholas Smedira and CRC Linda Clarke from the Cleveland Clinic.

Catheter-DCM: CRC Patti Mitchell from the Minneapolis Heart Institute Foundation, CRCs Patty Meldrum and Kristin Kolsch, and Dr Marco Costa and CRC Terrence Semenec from University Hospital Case Medical Center.

Data Safety Monitoring Board: Data safety monitoring board for both studies; members included Dr Warren Sherman (Chair), Dr Christof Stamm, Dr Ren-Ke Li, Richard McLain, and Susan Berman.

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DISCLOSURES

Timothy D Henry received institutional support and consultant fees as a Steering Committee member for an ongoing Aastrom Biosciences clinical trial. Amit N Patel received institutional support and consultant fees as a Steering Committee Chair for an ongoing Aastrom Biosciences clinical trial. Jay H Traverse, Cara East, Rodney S Badger, Baron L Hammon, Brian Bruckner and David A Bull all received institutional support for the conduct of this research. Ann E Remmers and David Recker are employees of Aastrom Biosciences, Inc.
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<td>Symptomatic, Functional, and Echocardiographic Measures</td>
<td>NYHA Class – n (%)</td>
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<tr>
<td>Combined</td>
<td>LVEF (%) Mean (SD)</td>
<td>Male</td>
<td>25.4 (10)</td>
<td>27.2 (7)</td>
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<td>29.3 (8)</td>
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<td>Catheter</td>
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<tr>
<td>Combined</td>
<td>LV EDV, 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>
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<td>229.2 (75)</td>
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<tr>
<td>Surgical</td>
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<td>234.3 (42)</td>
<td>202.4 (83)</td>
<td>0.38</td>
<td>219.6 (94)</td>
<td>219.6 (94)</td>
<td>0.82</td>
<td>225.7 (74)</td>
<td>217.2 (94)</td>
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<td>Catheter</td>
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<td>244.7 (57)</td>
<td>206.8 (60)</td>
<td>0.36</td>
<td>232.6 (114)</td>
<td>190.9 (74)</td>
<td>0.52</td>
<td>237.5 (86)</td>
<td>200.0 (64)</td>
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<tr>
<td>Combined</td>
<td>LV ESV, mL Mean (SD)</td>
<td>178.3 (45)</td>
<td>151.6 (60)</td>
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<td>169.0 (72)</td>
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<td>166.3 (38)</td>
<td>149.2 (74)</td>
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<td>167.5 (72)</td>
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<tr>
<td>Catheter</td>
<td></td>
<td>208.5 (60)</td>
<td>154.2 (46)</td>
<td>0.13</td>
<td>172.4 (87)</td>
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<td>186.8 (71)</td>
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<tr>
<td>Combined</td>
<td>6 Min Walk Distance, m</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>
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<td>380.4 (52)</td>
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<tr>
<td>Surgical</td>
<td></td>
<td>374.8 (14)</td>
<td>368.8 (69)</td>
<td>0.84</td>
<td>368.3 (58)</td>
<td>369.0 (105)</td>
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<tr>
<td>Catheter</td>
<td></td>
<td>409.3 (53)</td>
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<td>395.0 (91)</td>
<td>399.3 (84)</td>
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<td>402.2 (67)</td>
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<td>Combined</td>
<td>MLHFQ Global Score</td>
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<td>46.5 (23)</td>
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<td>Surgical</td>
<td></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>
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<td>49.4 (23)</td>
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<tr>
<td>Catheter</td>
<td></td>
<td>70.7 (24)</td>
<td>42.3 (23)</td>
<td>0.10</td>
<td>41.0 (26)</td>
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Table 2. Summary of Adverse Events Per Patient

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<th>Days 0-5</th>
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<tr>
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<tr>
<td>Surgical Study (IMPACT-DCM)</td>
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<td>Catheter Study (Catheter-DCM)</td>
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Table 3. Summary of Major Adverse Cardiac Events (MACE)

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<thead>
<tr>
<th>IMPACT-DCM (6 months)</th>
<th>Catheter-DCM (1 year)</th>
<th>Both Studies</th>
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<tbody>
<tr>
<td>Number/N (%) of Patients with a MACE</td>
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<td></td>
</tr>
<tr>
<td>Nonischemic</td>
<td>3/6 (50)</td>
<td>2/12 (17)</td>
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* Control vs. ixmyelocel-T p < 0.05 Fisher’s exact test

Table 4. Listing of MACE by Strata and Treatment Group

<table>
<thead>
<tr>
<th>MACE</th>
<th>Number/N (%) of Patients*</th>
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<th>Nonischemic</th>
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<tr>
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<td>Control</td>
<td>Ixmyelocel-T</td>
<td>Control</td>
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<tr>
<td>Cardiac death</td>
<td>1 (11)</td>
<td>1 (5)</td>
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</tr>
<tr>
<td>Cardiac arrest</td>
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<td>0 (0)</td>
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<tr>
<td>Myocardial infarction</td>
<td>0 (0)</td>
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<td>0 (0)</td>
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<tr>
<td>Ventricular arrhythmia†</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
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<tr>
<td>CHF exacerbation</td>
<td>4 (44)</td>
<td>0 (0)</td>
<td>3 (27)</td>
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<tr>
<td>Unstable Angina</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Major bleeding</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</table>

* 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 4.

† Two cases of ventricular arrhythmia occurred during the surgical procedure.
FIGURE LEGENDS

Figure 1. Patient Disposition. Eligible patients with DCM, stratified by ischemic/non-ischemic disease, were enrolled in two randomized open-label feasibility studies and followed for up to 24 months. In the IMPACT-DCM study, ixmyelocel-T was administered via mini-thoracotomy. In the Catheter-DCM study, ixmyelocel-T was administered with a NOGA Myostar injection catheter. The numbers of patients randomized, treated, and followed for 12 months post-treatment is shown for each study by strata. Patients randomized to control (standard of care) were given the opportunity to be rescreened after 6 months in the IMPACT-DCM study or after 12 months in the Catheter-DCM study and, pending eligibility, receive ixmyelocel-T. Safety and a limited number of efficacy assessments were evaluated in the study extensions.

Figure 2. Efficacy Assessments. Post-baseline efficacy assessments from patients from both studies are shown. The percentage of control patients (open circle) or ixmyelocel-T treated patients (filled circle) with a NYHA Class score of I or II is shown for A) ischemic and B) non-ischemic patients. Change from baseline in MLHFQ global score (mean and SEM) for control patients (open circle) or ixmyelocel-T treated patients (filled circle) is shown for C) ischemic and D) non-ischemic patients. Change from baseline in 6 minute walk distance (mean and SEM) for E) ischemic and F) non-ischemic patients. An asterisk (*) indicates statistically significant (p < 0.05) differences between groups.

Figure 3. Cardiac Structure and Function. Change from baseline in LVEF (%) is shown for control patients (open circle) or ixmyelocel-T treated patients (filled circle) for A) ischemic and B) non-ischemic patients. Change from baseline in LV end-systolic volume is shown for control patients (open circle) or ixmyelocel-T treated patients (filled circle) for A) ischemic and B) non-ischemic patients. Change from baseline in LV end-diastolic volume is shown for control patients (open circle) or ixmyelocel-T treated patients (filled circle) for A) ischemic and B) non-ischemic patients. There were no statistically significant differences (p < 0.05) between groups.

Figure 4. SPECT-Derived Wall Motion Score Index. Baseline and Month 6 follow-up SPECT-derived wall motion score index values for 14 ixmyelocel-T treated ischemic DCM patients (ix-T) or 5 ischemic DCM control patients from both studies are shown in A); mean (+SEM) values are shown in B). Only SPECT data from optimal baseline and month 6 images of diagnostic quality are included. There was no statistically significant differences (p < 0.05) between baseline and month 6 mean wall motion score index values.
Novelty and Significance

What Is Known?

- Results of clinical trials with bone marrow mononuclear cells (BMMCs) 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 multi-cellular therapeutic agent cultured from autologous BMMCs that contain approximately 200-fold and 50-fold the number of M2-like macrophages and mesenchymal stem cells (MSCs) than BMMCs, respectively.

What New Information Does This Article Contribute?

- In two 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 non-ischemic 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 BMMC for the treatment of heart failure has been shown to be well tolerated but, efficacy is limited. As a potential improvement to treatment with autologous BMMCs, ixmyelocel-T, an expanded and enhanced stem cell therapy agent was evaluated for the treatment of ischemic and non-ischemic DCM. Two randomized, open label studies were conducted to evaluate the safety and feasibility of two methods of ixmyelocel-T cell delivery as well as to identify potential clinical benefit. Improvement in NYHA Class, MLHFQ score, and 6-minute walk distance was observed in the ischemic patients starting 1 month after treatment and sustained through 12 months. In addition, the number of MACE was lower in the treated ischemic DCM patients compared with ischemic control DCM patients. Similar improvements were not observed in the non-ischemic 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 DCM patients.
Figure 1. Patient Disposition

Eligible patients with DCM, stratified by ischemic/non-ischemic disease, were enrolled in two randomized open-label feasibility studies and followed for up to 24 months. In the IMPACT-DCM study, tirofiban 1 was administered via me-too-thrombolytics. In the Catheter-DCM study, tirofiban 1 was administered with a NgA MioStar injection catheter. The numbers of patients randomized, treated, and followed for 12 months post-treatment is shown for each study by strata. Patients randomized to control (standard of care) were given the opportunity to be rescreened after 6 months in the IMPACT-DCM study or after 12 months in the Catheter-DCM study and, pending eligibility, receive tirofiban 1. Safety and a limited number of efficacy assessments were evaluated in the study extension.
Figure 2. Efficacy Assessments

Post-baseline efficacy assessments from patients from both studies are shown. The percentage of control patients (open circle) or enzalutamide-treated patients (filled circle) with a NYHA Class score of I or II is shown for (A) ischemic and (B) non-ischemic patients. Change from baseline in MLFDQ global score (mean and SEM) for control patients (open circle) or enzalutamide-treated patients (filled circle) is shown for (C) ischemic and (D) non-ischemic patients. Change from baseline in 6-minute walk distance (mean and SEM) for (E) ischemic and (F) non-ischemic patients. An asterisk (*) indicates statistically significant (p < 0.05) differences between groups.
Figure 3. Cardiac Structure and Function

Change from baseline in LVEF (%) is shown for control patients (open circle) or ischemic-T treated patients (filled circle) for A) ischemic and B) non-ischemic patients. Change from baseline in LV end-diastolic volume is shown for control patients (open circle) or ischemic-T treated patients (filled circle) for A) ischemic and B) non-ischemic patients. Change from baseline in LV end-diastolic volume is shown for control patients (open circle) or ischemic-T treated patients (filled circle) for A) ischemic and B) non-ischemic patients. There were no statistically significant differences (p < 0.05) between groups.
Figure 4. SPECT-Derived Wall Motion Score Index

Baseline and Month 6 follow-up SPECT-derived wall motion score index values for 14 ischaemic-T
related ischaemic DCM patients (n=7) or 5 ischaemic DCM control patients from both studies are shown in
A: mean ± (SEM) values are shown in B. Only SPECT data from optimal baseline and month 6 images
of diagnostic quality are included. There was no statistically significant difference (p < 0.05) between
baseline and month 6 mean wall motion score index values.
Safety and Efficacy of Ixmyelocel-T: An Expanded, Autologous Multi-Cellular Therapy, in Dilated Cardiomyopathy
Timothy D Henry, Jay H Traverse, Baron L Hamman, Cara A East, Brian Bruckner, Ann E Remmers, David Recker, David A Bull and Amit Patel

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

#### Supplemental Material

**Supplemental Table**

**Summary of Deaths, LVAD placements, and Heart Transplants**

<table>
<thead>
<tr>
<th></th>
<th>IMPACT-DCM (6 months)</th>
<th>Catheter-DCM (1 year)</th>
<th>Both Studies</th>
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<tr>
<td></td>
<td>Control</td>
<td>Ixmyelocel-T</td>
<td>Control</td>
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<td>Number/N (%) of Deaths</td>
<td>1/14 (7)</td>
<td>2/24 (8)</td>
<td>0/6 (0)</td>
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<tr>
<td>Ischemic</td>
<td>1/6 (17)</td>
<td>2/12 (17)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>0/8 (0)</td>
<td>0/12 (0)</td>
<td>0/3 (0)</td>
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<td>Number/N (%) of Heart Transplants</td>
<td>0/14 (0)</td>
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<td>Number/N (%) of LVAD placements</td>
<td>1/14 (7)</td>
<td>3/24 (13)</td>
<td>1/6 (17)</td>
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<td>Ischemic</td>
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<td>0/12 (0)</td>
<td>0/3 (0)</td>
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<tr>
<td>Nonischemic</td>
<td>1/8 (15)</td>
<td>3/12 (25)</td>
<td>1/3 (33)</td>
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</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 ≥3,000/μL
    • Absolute neutrophil count ≥1,500/μL
    • Platelets ≥140,000/μL
    • AST (SGOT)/ALT (SGPT) ≤2.5 X institutional standards range
    • Creatinine ≤2.5 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 ≤140 and a diastolic blood pressure of ≤90 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:
    o 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.
    o Use of ACE inhibitors and/or AT-1 receptor blockers as well as loop diuretics (e.g., furosemide).
    o 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 metoprolol 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 (≥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 ≤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 ≥3,000/μL
    - Absolute neutrophil count ≥1,500/μL
    - Platelets ≥140,000/μL
    - AST (SGOT)/ALT (SGPT) ≤2.5 X institutional standards range
    - Creatinine ≤ 2.5 mg/dL

11. Controlled blood pressure (defined as a systolic blood pressure ≤140 and a diastolic
    blood pressure of ≤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
      pacers/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 \textit{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
