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The ixCELL-DCM Trial: Rationale and Design

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Running Header: Design of the ixCELL-DCM trial

ABSTRACT

Ixmyelocel-T is an investigational patient-specific, expanded, multicellular therapy produced from a patient's own bone marrow. It is produced by selectively expanding two key types of bone marrow mononuclear cells, CD90+ mesenchymal stem cells and CD45+CD14+ autofluorescent alternatively activated macrophages. Earlier clinical trials suggested that intramyocardial ixmyelocel-T might improve clinical, functional, symptomatic, and quality of life outcomes in patients with ischemic dilated cardiomyopathy (IDCM). This ongoing randomized, double-blinded, placebo-controlled phase 2b trial (ixCELL-DCM) was designed to assess the efficacy, safety, and tolerability of catheter-based transendocardial injection of ixmyelocel-T in patients with heart failure due to IDCM. Patients (N = 114) with New York Heart Association class III or IV symptomatic heart failure due to IDCM, who have left ventricular ejection fraction ≤ 35% and an automatic implantable cardioverter defibrillator, but are ineligible for revascularization procedures, were randomly assigned (1:1 ratio) to ixmyelocel-T or placebo (vehicle control). The primary efficacy endpoint is a composite of the total number of deaths, cardiovascular hospitalizations, or unplanned clinic visits to treat acutely decompensated heart failure during the 12 months following treatment administration. Secondary endpoints include the win ratio analysis for hierarchical occurrences of clinical events in the primary endpoint, total numbers of clinical events, left ventricular structure and function, and quality-of-life assessments. IxCELL-DCM is one of the largest cell therapy trials in heart failure patients to date and the first double-blinded, placebo-controlled study of ixmyelocel–T administered via transendocardial catheter-based injections in patients with heart failure secondary to IDCM.

KEYWORDS: heart failure, ischemic dilated cardiomyopathy, ixmyelocel-T, stem cell therapy

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INTRODUCTION

Heart failure (HF) continues to be a major cause of morbidity and mortality in the United States (U.S.) and throughout the world (12,19,28). Despite advances in both pharmacologic and device therapy, mortality from HF continues to approach 50% within 5 years (10). In addition, the prevalence of HF is growing as the mortality from coronary artery disease has improved and the population ages. Despite optimal pharmacologic and device therapy many patients are, therefore, left with limited treatment options beyond left ventricular assist device and/or cardiac transplantation (12,28).

In 2001, Orlic et al demonstrated in a mouse myocardial infarction model that intramyocardial injection of bone-marrow derived stem cells can lead to myocardial regeneration (21,22). This landmark finding stimulated interest in cellular therapies for HF. Based on the results of positive pre-clinical studies and promising initial clinical trials, cell therapy appears to be an attractive alternative therapy (5,6,9,23,31). Initial clinical trials focused on autologous bone marrow mononuclear cells (BMMC) (6,9,31). Following a number of encouraging early trials, the NIH-sponsored cardiovascular cell therapy network (CCTRN) published the FOCUS trial which demonstrated no improvement in maximal oxygen consumption or in left ventricular end systolic volume (LVESV) in 92 patients with ischemic cardiomyopathy who underwent intramyocardial delivery of 100 million autologous BMMC (26). However, there was a significant (2.7%) improvement in left ventricular ejection fraction (LVEF). Consistent with the known age and risk factor-related decline in the number and potency of autologous BMMC, the benefit was found to be related to the patient's age as well as the specific cell composition (higher CD34+ or CD133 cell counts) (26). The modest improvement seen with autologous BMMC has stimulated interest in a new generation of cell therapies to improve clinical outcome. Novel approaches which have been utilized include specific cell populations such as

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mesenchymal stem cells (MSC) (13,18), CD34+ stem cells (30), or adipose derived stem cells (25), the use of allogeneic cells (11, 24), the use of cardiac derived cells (4,17), or a combination of those approaches.

Because BMMCs isolated from bone marrow include very few regenerative cells, and a large number of BMMCs are required to induce angiogenesis (16), another approach is to expand or enhance autologous bone marrow-derived cells prior to intramyocardial injection. Autologous bone marrow derived MSCs enhanced in a 'cardiogenic cocktail' were tested in the phase 2 C-CURE trial (2) and ixmyelocel-T was tested in the IMPACT-DCM and CATHETER-DCM phase 2a trials (14).

Ixmyelocel-T is an autologous, bone marrow-derived, multicellular therapy produced by expanding 2 key types of BMMCs, CD90+ MSCs and CD45+CD14+ autofluorescent (CD14+ Auto+) M2 (alternatively activated) macrophages, using proprietary cell processing technology (1). (Figure 1) Ixmyelocel-T contains the same mixture of cell types found in the BMMC population, but approximately 200 times the number of M2 macrophages and 50 times the number of CD90+ MSCs. Because it is a mixed-cell therapy, ixmyelocel-T is believed to have a wider range of biological activities than a single-cell therapy (1). Preclinical studies have shown that the biological activities of ixmyelocel-T include tissue remodeling, immunomodulation, angiogenesis, and endothelial protection (1,16).

The open-label phase 2a trials IMPACT-DCM and CATHETER-DCM examined the safety and efficacy of intramyocardial injection of ixmyelocel-T in patients with ischemic or non-ischemic dilated cardiomyopathy (DCM), compared to a standard of care control group (14). Ixmyelocel-T was injected into the myocardium via thoracotomy in IMPACT-DCM and via catheter-based transendocardial injections in CATHETER-DCM. In pooled data from the 2

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trials, 21 ischemic DCM (IDCM) and 18 non-ischemic DCM patients were treated with ixmyelocel-T. More serious adverse events (SAEs) were observed when ixmyelocel-T was administered via surgery compared to catheter-based transendocardial administration. Among IDCM patients, treatment with ixmyelocel-T was associated with fewer major adverse cardiovascular events (MACE) and improvement in New York Heart Association (NYHA) class, 6-minute walk test distance (6MWT), and Minnesota Living with HF Questionnaire (MLHFQ). Similar benefits were not observed in the patients with non-ischemic dilated cardiomyopathy. Because of these results, this larger phase 2b trial was designed to include only IDCM patients, using catheter-based transendocardial delivery of ixmyelocel-T.

METHODS

Study Design

ixCELL-DCM was a phase 2b, randomized, double-blinded, placebo-controlled, parallel-group trial conducted at 31 sites in North America which began enrollment October 2012 (ClinicalTrials.gov identifier: NCT01670981) and is ongoing in 2016. The primary objective was to assess the efficacy, safety, and tolerability of ixmyelocel-T compared to placebo, administered via transendocardial catheter-based injections, in patients with end-stage HF due to IDCM who were ineligible for revascularization procedures. The primary analysis will be performed 12 months following administration of study treatment. The trial was conducted in accordance with ICH Good Clinical Practice guidelines and with the approval of the Investigational Review Board at each site. A list of the institutions that enrolled subjects is provided in the online Appendix. All study subjects provided signed informed consent prior to study entry. The informed consent stated that the treatment was experimental and that there was no subject payment for treatment.

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Study Population

This trial enrolled adult men and women with symptomatic NYHA class III or IV HF due to IDCM, with LVEF ≤35%, who have been implanted with an automatic implantable cardioverter defibrillator (AICD) but were ineligible for revascularization procedures. Complete inclusion and exclusion criteria are listed in Tables 1 and 2. In order to enrich the study population with patients expected to experience HF-related clinical events during the 12-month trial, eligible patients must have had a HF-related hospitalization or unscheduled outpatient or ED visit within 6 months prior to screening (15). Due to slow early enrollment in the trial, that inclusion criterion was modified to include patients who had either a recent HF-related hospitalization within 6 months of screening, an elevated B-type natriuretic peptide (BNP) (≥400 pg/mL) or N-terminal prohormone BNP (NT-proBNP) ≥2000 pg/mL) within 30 days of or at screening, or reduced 6MWT (<400 m) at screening. Recent hospitalization for HF, elevated BNP, or NT-proBNP, and reduced 6MWT have been shown to correlate with increased likelihood of death or cardiovascular events (3,7,20). Other changes made to the original inclusion criteria to improve enrollment but not compromise the study conduct, included an increase in the baseline LVEF requirement from ≤30% to ≤35%, and allowing the enrollment of patients who had received gene therapy >12 months prior to screening.

Patients who met eligibility criteria were randomly assigned to the ixmyelocel-T or placebo (vehicle control) group (1:1 ratio), with randomization stratified by study site. At baseline, all patients underwent protocol assessments including: physical examination, NYHA classification, laboratory tests, immune response blood sample collection, AICD interrogation, 12-lead electrocardiogram (ECG), echocardiogram, single-photon emission computed

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tomography (SPECT) rest/stress imaging, 6MWT, MLHFQ, EuroQoL-5D (EQ-5D) Questionnaire, and medical resource utilization (MRU) data collection.

Bone Marrow Aspiration and Ixmyelocel-T Preparation

All randomized patients, including those assigned to placebo, underwent a percutaneous, small volume (~60 mL) bone marrow aspiration from the posterior iliac crest under appropriate anesthesia. Bone marrow aspirate was shipped overnight to a central manufacturing facility (Vericel Corporation [formerly Aastrom Biosciences, Inc.], Ann Arbor, MI). If the initial bone marrow aspirate did not meet established specifications for viability or cellularity necessary to manufacture ixmyelocel-T (Table 3), a second bone marrow aspiration was allowed, up to 30 days after randomization. If a second aspirate was not collected, or if the second aspirate also did not meet established specifications, the patient was withdrawn from the trial and replaced.

The ixmyelocel-T manufacturing process is initiated by using an automated, closed system (SEPAX Cell Separation System [Biosafe, Houston, TX, USA]) to perform a Ficoll-based density gradient centrifugation process to deplete red blood cells and purify bone marrow mononuclear cells (BMMNCs). The purified BMMNCs are collected and transferred into a single-use, sterile, disposable cell cassette that is a component of Vericel's proprietary, automated, closed cell processing system. The system uniformly distributes the cells over the culture surface and then controls the culture conditions, including temperature, culture medium exchange, and gas exchange which results in the expansion of the CD90+ and CD14+Auto+ cells as described previously (1). The bone marrow aspirate was cultured for 12±1 days using proprietary cell processing technology, and then harvested and formulated as the final product, ixmyelocel-T. Ixmyelocel-T preparations containing 40 – 200 x 10^6 cells with ≥ 85% viability

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that met all release specifications described in Table 3 were shipped overnight in a temperature-controlled shipping container to the study site for injection.

Bone marrow aspirate from patients randomized to placebo was also cultured and expanded into ixmyelocel-T and tested against release specifications. In order to maintain the double-blinded validity of the trial, if ixmyelocel-T preparations for patients in the placebo group did not meet release specifications, that patient had the option of undergoing a second bone marrow aspiration to remain in the trial; if not, that patient was withdrawn from the trial and replaced. A vehicle control composed of the same excipients as used in ixmyelocel-T (approximately 67% HypoThermosol with the remainder isolyte supplemented with 0.25% human serum albumin) was prepared for all patients in the placebo group at the centralized manufacturing facility.

Ixmyelocel-T or placebo was shipped to each site in a validated temperature-controlled container to maintain a temperature of 0°C-12°C. The product has a maximum 72-hour "cold temperature" shelf life, which starts at the last processing step at the manufacturing facility. After removal from the shipping container, the product has a 2-hour ambient temperature shelf life, within which it must be prepared and injected into the patient.

Pharmacists (or other designated study personnel) loaded 0.8 mL of the final product into 1-mL syringes, allowing two 0.4-mL injections per syringe. Ixmyelocel-T and placebo have different physical characteristics, which may be evident to study personnel who handle the shipped product or syringes. Therefore, study personnel who prepared the syringes, and the physician and any assistant(s) involved in administering injections, were considered unblinded and not allowed to perform follow-up procedures or assessments of study patients after injection. A separate team of study personnel, who were not involved in syringe preparation or injection

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administration, were considered blinded to study treatment and performed all post-injection follow-up safety and efficacy assessments.

Cardiac Mapping and Transendocardial Injections

Approximately 15 days after bone marrow aspiration, cardiac mapping and transendocardial injections of ixmeylocel-T or placebo were performed using the NOGA® XP Cardiac Navigation System (Biosense Webster, Diamond Bar, CA), by investigators with certified training on that system. Patients were prepared for cardiac catheterization according to each site's standard procedures. First, using the NOGA-STAR® catheter (Biosense, Inc.), a series of individual points within the left ventricle were recorded and tracked to generate a 3-dimensional electromechanical map of its inner contours, helping to identify the border between viable and non-viable myocardium. Then the MYOSTAR® injection catheter (Cordis Corporation) was used to inject either ixmyelocel-T or placebo into target tissue. The target tissue for injections was viable tissue, defined as having unipolar voltage ≥4 mV. Injections were performed according to the following guidelines: (1) No injections near the mitral valve annulus or the apex of the heart; (2) The catheter tip is perpendicular to the myocardial wall; (3) Loop stability ≤3 mm; (4) Needle length ≤50% of LV wall thickness; (5) Avoid significant (>1 mV) ST elevation on unipolar intracardiac electrogram during catheter positioning. After the needle was inserted into the myocardium ideally, a premature ventricular contraction was observed.

Each patient received approximately 12 to 20 0.4-mL injections, depending on the final product volume. Injections were made into viable tissue only and spaced approximately ≥1.0 cm apart. Ideally, injections were evenly distributed along the "border" between viable and non-viable myocardium. After the "border" zone was treated, any additional injections were evenly distributed across viable myocardium. If a clearly delineated border was not present, injections

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were made into viable tissue, preferably in areas known to be ischemic (e.g., identified by prior imaging). Injections were administered at a rate of at least 15 seconds for each 0.1 mL of volume; each 0.4-mL injection was administered over at least 60 seconds. The needle remained in the myocardium for approximately 5-10 seconds after each injection to minimize backflow from the injection site.

The injection procedure was terminated if at any time the patient experienced an AE, such as: persistent chest pain; sustained hypotension unresponsive to intravenous fluid administration and one inotrope; shortness of breath; any unanticipated change in level of consciousness or neurological status; acute coronary syndrome; suspected or confirmed cardiac tamponade; recurrent, hemodynamically unstable, sustained runs of ventricular tachycardia or ventricular fibrillation requiring cardioversion; new thrombus in the left ventricle or the aorta; suspected or confirmed aortic dissection; or cerebral vascular accident. In addition, the procedure was terminated if there was any uncertainty about the location of the catheter tip in relation to the vasculature or the left ventricle. Following the catheterization and injection procedure, patients were hospitalized overnight to monitor for any complications. An echocardiogram was performed soon after intramyocardial injections and 4-6 hours later to confirm the absence of a pericardial effusion, and cardiac biomarkers (CK, CK-MB, and troponin I) were assessed 6 and 12 hours after injection and prior to hospital discharge to assess any myocardial injury.

Follow-up Assessments and Open-Label Extension

Follow-up evaluations of all patients will be performed at 1, 3, 6, and 12 months following injection (study day 1). Follow-up assessments include: physical examination, NYHA classification, laboratory tests, blood collection for subsequent immune response testing, monitoring of AEs and concomitant medications, catheter site monitoring, AICD interrogation,

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12-lead ECG, echocardiogram, 6MWT, MLHFQ, EQ-5D, and MRU data collection. All hospitalizations, outpatient or emergency department (ED) visits, and deaths that occur after treatment will be documented. For each event, the underlying cause, any available clinical diagnostic information, and management will be recorded by the site investigator and/or treating clinician. This information will be reviewed by an independent adjudication team. An independent Data and Safety Monitoring Board reviewed unblinded safety data periodically throughout the trial.

After 12-month assessments have been collected for all patients, primary data analysis will be performed and the trial blind will be broken. If the 12-month data analysis demonstrates clinically meaningful benefit of ixymelocel-T over placebo, patients in the placebo group will have the option to receive ixmyelocel-T open-label and continue in an open-label extension. The objective of the open-label portion of the study is to assess safety of ixmyelocel-T. Patients who do not continue into the open-label portion of the study will be followed with a phone call at month 24 to track any SAEs. The trial was originally planned to maintain the double blind for 24 months, but it was changed to give patients in the placebo group the opportunity to receive ixmyelocel-T after all patients have completed the first 12 months of the trial and if evidence of clinical benefit is established.

Endpoints

As discussed earlier, the mixed-cell composition of ixmyelocel-T is hypothesized to contribute to a range of biological activities involved in repair and regeneration in ischemic tissue. Efficacy will be assessed by the incidence of clinical events (deaths, hospitalizations, and outpatient and ED visits) and functional, structural, and symptomatic/quality of life outcomes.

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Clinical events will be adjudicated by an independent review committee of experts who were not involved in the trial and are blinded to treatment.

The primary efficacy endpoint of ixCELL-DCM is a composite clinical event endpoint comprised of the total number of deaths, cardiovascular hospitalizations, or unplanned outpatient and ED visits to treat acutely decompensated HF (ADHF) during the 12 months following administration of study treatment, excluding events considered related to the administration procedure (Table 4). This primary endpoint was chosen because the US Food and Drug Administration (FDA) recommends that phase 3 trials of cardiovascular cell therapies assess efficacy with clinical event endpoints (i.e. LVEF) (29). A surrogate primary endpoint, not driven by clinical events, was considered for this phase 2b trial, however, it was decided that a positive result on a clinical event endpoint, although more challenging to achieve in a relatively small trial, would provide stronger evidence of efficacy and would more accurately predict the outcome of a subsequent, larger phase 3 trial.

A key secondary efficacy endpoint is the win ratio of hierarchical occurrences of the clinical events included in the primary endpoint (27) over 12 months. In a composite clinical event endpoint, events are considered to be of equal clinical significance. A win ratio, on the other hand, takes into account that death, VAD implant, or heart transplant, is more clinically significant than a hospitalization, which is more clinically significant than an unplanned outpatient or ED visit to treat acutely decompensated HF. Patients in the ixmyelocel-T group will be compared to placebo patients first to determine which patient died earlier (i.e., had shorter time from randomization to death) or had a VAD implant or heart transplant. The ixmyelocel-T patient "wins" if the time from randomization to death/VAD/heart transplant is shorter for the placebo patient than the ixmyelocel-T patient, across the same duration of follow-up. If neither

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patient wins, their time to cardiovascular hospitalization will be compared. If they are still tied, their time to an unplanned outpatient or ED visit to treat ADHF will be compared. The win ratio is then calculated as the total number of wins for ixmeylocel-T across all events compared with the total number of wins for placebo. Pocock and colleagues recommended comparing matched pairs of patients, one from the experimental group and one from the control group. ixCELL-DCM instead will use the unmatched approach by Finkelstein and Schoenfeld to compare all patients in the ixmyelocel-T group to all patients in the placebo group (8).

Other secondary efficacy endpoints will assess function (6MWT), LV structure (LVEF, LV volumes, and LV wall motion on echocardiogram), and symptoms/quality of life (NYHA class, MLHFQ, and EQ-5D), at 3, 6, and 12 months. Additional secondary efficacy endpoints include the total number of days hospitalized, time to onset of clinical events, effect of treatment on biomarkers, and the incidence of ventricular arrhythmias. Secondary safety endpoints assess safety and tolerability of ixmyelocel-T from the time of aspiration through the 24 months after administration, as well as any immune response where blood will analyzed for presence of antibovine and antiequine antibodies for up to 3 months following treatment.

Statistical Analysis

The primary endpoint will compare the total number of clinical events in the ixmyelocel-T group compared to placebo 12 months after injection, using Poisson regression to estimate the incidence rate ratio. A sample size of approximately 114 patients (randomized 1:1 to ixmyelocel-T or placebo) will provide 82% power to detect a treatment difference at a two-sided 0.05 significance level (assuming event rates per patient year of 1.3 with placebo and 0.7 with ixmyelocel-T). Events considered related to injection administration will not be counted in the primary analysis. One of the components of the primary endpoint was changed from all-cause

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hospitalization to cardiovascular hospitalization in the primary endpoint, since reduction in cardiovascular hospitalization is more linked to the presumed mechanism of ixmyelocel-T than is all-cause hospitalization. In addition, the analytical approach was changed to Poisson regression because deaths that occurred soon after treatment resulted in large individual event rates and greater than expected variability that is more appropriately assessed using Poisson regression.

All efficacy analyses will use a two-sided 0.05 significance level and be performed on the full analysis set, defined as a modified intent-to-treat set of patients who were randomized and aspirated and received all planned injections of their randomized treatment. Secondary efficacy endpoints related to incidence of clinical events will be analyzed similarly to the primary endpoint, but will include events related to treatment administration. Secondary efficacy endpoints related to change from baseline will be analyzed using analysis of covariance, with treatment as a factor and the baseline value as a covariate, to determine least squares means (LSM), differences in LSMs compared to placebo, and 95% confidence intervals. If the specified assessment data are missing, the last non-missing, post-baseline assessment will be carried forward. Secondary endpoints relating to time to events will be summarized with Kaplan-Meier survival curves and analyzed using a Cox proportional hazards model to determine the hazard ratio, its 95% CI, and p-value. Given the large number of secondary endpoints, p-values for all secondary endpoints will be considered descriptive.

All secondary safety analyses will be performed on the safety set, the set of all randomized patients who received study treatment. AEs will be summarized by system organ class, severity, and relationship, and by treatment group and overall. AEs will be documented from the date of signing the informed consent document until month 12 or early termination, but only aspiration-emergent (occurring between aspiration and injection) or treatment-emergent

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(occurring during or after injection) AEs will be reported. Only SAEs will be documented between months 12 and 24.

Committees

The steering committee for ixCELL-DCM, an international group of experts in heart failure trials and in cardiac cellular therapies (listed in the online Appendix), provided significant guidance for the design of the trial and in monitoring the trial's progress.

Safety of ixCELL-DCM is being monitored by an independent Data and Safety Monitoring Board. An additional committee, independent of the other committees and based at Brigham and Women's Hospital in Boston, MA, will adjudicate clinical events in the primary and secondary endpoints.

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DISCUSSION

ixCELL-DCM is the first double-blinded, placebo-controlled study of ixmyelocel–T administered via transendocardial catheter-based injections in patients with HF secondary to IDCM and will be the largest randomized trial of cellular therapy for HF due to ischemic cardiomyopathy to be presented at this time.

The randomized, placebo-controlled, double-blinded design of ixCELL-DCM is one of its strengths. Patients randomly assigned to placebo underwent bone marrow aspiration and transendocardial injections just as patients assigned to ixmyelocel-T. In the phase 2a ixmyelocel-T trials, only treated patients underwent bone marrow aspiration and intramyocardial injection (14), similar to a number of the previously published trials –Phase 1 FOCUS, Mesoblast, C-Cure (31,24,2). Ixmyelocel-T and placebo have different physical characteristics, making it impossible to blind interventionalists to treatment, therefore each study site was staffed with an additional blinded team to make post-treatment assessments.

Other strengths include the central evaluation of echocardiograms, both at screening to determine whether study subjects met the LVEF inclusion criterion, and also during the trial, to detect changes in LVEF and LV structure (secondary endpoints). The trial also benefited from guidance by its steering committee, which recommended changes to the trial design when early enrollment in the trial was slow, and by close monitoring of safety by an independent Data and Safety Monitoring Board. Additionally, an independent, blinded adjudication team reviewed clinical events that will become part of the efficacy analyses.

Limitations of ixCELL-DCM include its relatively modest size, which makes it a proof-of-concept or feasibility study, in particular because the primary endpoint involves differences in clinical events. To overcome this limitation and to increase the likelihood of patients having a

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clinical event, patients are required to have a HF hospitalization (or unplanned outpatient or ED visit to treat ADHF) within the prior 6 months, or an elevated BNP or NT-proBNP or a 6MWT <400 meters. Another potential limitation is that magnetic resonance imaging, potentially the most accurate imaging technique for detecting LV structural changes (a secondary endpoint), was felt to be supoptimal because patients were required to have AICD's and because 10-15% of patients in trials using magnetic resonance imaging have been unable to obtain follow-up images.

Administering the placebo in ixCELL-DCM via transendocardial injection poses a potentially greater risk to patients than would be expected in the placebo arm of a drug trial. Because of this greater risk, the FDA's guidance on cardiac cellular therapies recommends allowing placebo patients to receive active treatment after the study period has ended, if efficacy of the active treatment has been conclusively demonstrated (29). Therefore, patients in the placebo arm have the option of receiving active treatment if the 12-month data analysis establishes efficacy of ixmyelocel-T.

One of the more unique aspects of ixCELL-DCM is prospectively designating win ratio as a secondary endpoint. In a 2012 article, Pocock et al recommended using a win ratio to emphasize the more clinically important component(s) of a composite endpoint (27). The components of the primary endpoint in ixCELL-DCM are of varying importance, so a win ratio is appropriate for this trial. ixCELL-DCM is, as far as we know, one of the first trials cardiovascular cell therapy trials to prospectively designate a win ratio as an endpoint.

Ixmyelocel-T differs from other cardiac cellular therapies because it contains a complete range of BMMC cell types, but with selective expansion of CD90+ MSCs and CD45+CD14+ autofluorescent alternatively activated macrophages. ixCELL-DCM will inform whether

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intramyocardial injection of this expanded, multicellular therapy may improve clinical outcomes among patients with HF secondary to IDCM.

In conclusion, cell therapy may be an attractive alternative for class III/IV HF patients who have exhausted pharmacological and device treatment options. While the initial clinical trials using autologous BMMC showed excellent safety, efficacy was modest due to the age and risk factor related decline in stem cell number and potency. This has stimulated a number of new approaches to enhance the therapeutic efficacy. IxCell-DCM will represent the largest randomized placebo-controlled trial of this new generation of cell therapies to be presented in 2016.

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Figure Legend

Figure 1. Production of Ixmyelocel-T

Ixmyelocel-T is composed of a mixture of cell types that include those expected to be found in the BMMC population. These include myeloid cells (granulocytes, monocytes, and mixed myeloid progenitors) and lymphoid cells (T cells, B cells, and mixed lymphoid progenitors) that express CD45 on the cell surface, CD90+ MSCs, and CD45+CD14+ autofluorescent+ (CD14+Auto+) macrophages. The numbers of CD90+ and CD14+Auto+ cells are significantly greater in ixmyelocel-T due to expansion during the Aastrom (now Vericel) proprietary expansion process.

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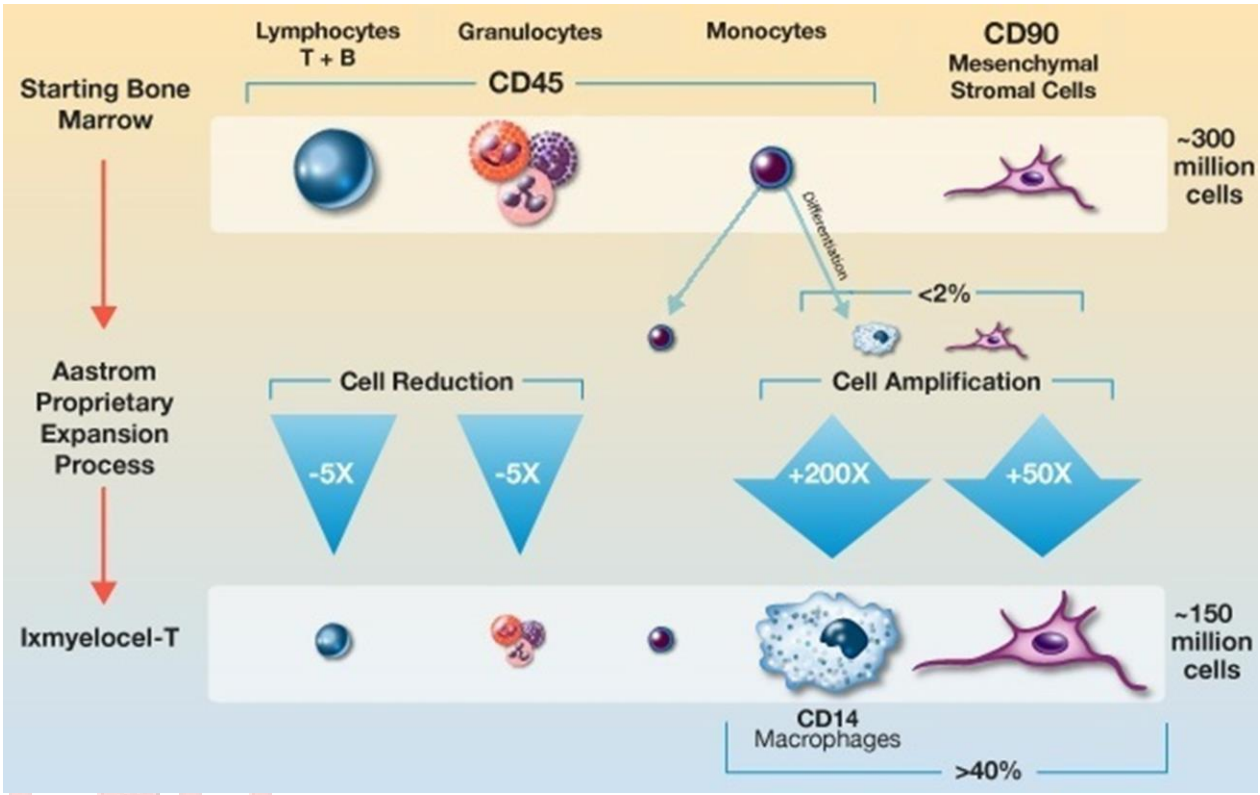

Figure 1

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Table 1. Inclusion Criteria

Male or non-pregnant, non-lactating female
30 to 86 years of age
Diagnosis of IDCM according to WHO criteria
LVEF ≤35% by echocardiogram [a]
NYHA functional class III or IV HF [b]
Not a candidate for reasonable surgical or percutaneous interventional revascularization procedures that will produce clinical improvement [c]
Receiving appropriate therapy for HF per clinical standard of care, for at least 30 days prior to screening • No new continuous-use medications introduced or discontinued in the 30 days prior to screening
Has an AICD implanted at least 3 months prior to screening
At least one of the following: • HF-related hospitalization within 6 months • Unplanned outpatient or ED visit to treat ADHF within 6 months (can include treatment with intravenous medication [diuretic, inotrope or vasoactive] or mechanical fluid removal) • BNP ≥400 pg/mL or NT-proBNP ≥2000 pg/mL within 30 days of or at screening • 6MWT distance ≤400 meters at screening
Life expectancy >12 months [b]
LV wall thickness ≥7 mm by echocardiogram at anticipated target injection area [b]
Hemodynamic stability without IV vasopressors or support devices
Acceptable candidate for bone marrow aspiration, cardiac catheterization, and transendocardial injection [b]
Willing and able to comply with scheduled study visits and to tolerate study procedures
Voluntarily sign an informed consent document

[a] LVEF values were determined by a central imaging core lab

[b] Investigator-determined

[c] Investigator-determined, based on coronary angiography. This opinion was reviewed and confirmed by a cardiac surgeon and an interventional cardiologist

Abbreviations: 6MWT, 6-minute walk test; ADHF, acutely decompensated HF; BNP, brain (or B-type) natriuretic peptide; ED, emergency department; IDCM, ischemic dilated cardiomyopathy; LV, left ventricular; NT-pro-BNP, amino-terminal prohormone BNP; NYHA, New York Heart Association; WHO, World Health Organization.

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Table 2. Exclusion Criteria

Disease-specific • Severe primary valvular heart disease or aortic valve prosthesis • VAD implantation, heart transplantation, cardiomyoplasty, LV reduction surgery, or cardiac shunt implantation • Planned HF-related device intervention or planned cardiac procedures • Subjects on heart transplant list who are status 1A or 1B • Current arrhythmias that would reduce accuracy of NOGA® electromechanical mapping and NOGA®-guided injection • LV thrombus • Myocardial infarction within 3 months prior to screening • Percutaneous coronary intervention, valvuloplasty, cardiac surgery, or other major cardiac procedure within 30 days prior to screening • LV wall unsuitable for transendocardial injections, due to thickness or other reason
Medical history • Stroke or transient ischemic attack within 3 months of screening • Hemoglobin A_{1c} (HbA_{1c}) ≥9% • Among diabetic patients, uncontrolled or untreated proliferative retinopathy as determined by dilated eye exam administered by a qualified eye care professional • Blood clotting disorder not caused by medication (eg, thrombophilia) • Active malignancy (non-basal cell) including those requiring surgery, chemotherapy, and/or radiation in the past 12 months; • Drug or alcohol abuse that would interfere with the compliance with study procedures • Known allergies to any equine, porcine, or bovine products • Body mass index ≥40 kg/m^2 • Established chronic kidney disease requiring dialysis (Stage 5) or estimated creatinine clearance <15 mL/min • Allergic to or unable to tolerate imaging contrast agents or the inability to get a good quality echocardiogram image at screening (as determined by the imaging core lab)
Abnormal laboratory values • Platelets <50,000/μL • Hemoglobin <9.0 g/dL • Aspartate aminotransferase/alanine aminotransferase >3 times the upper limit of normal • Human immunodeficiency virus-1 (HIV-1), HIV-2, or syphilis positive • Active hepatitis B surface antigen (HBsAg) or active hepatitis C virus (HCV) antibodies
Exclusionary procedures, devices, or medication • Receiving anti-angiogenic agents • Chronic exposure to cytotoxic therapy or radiation therapy within 3 months prior to screening or over the course of the trial • Concurrent participation in another interventional clinical trial or receiving experimental intervention within 30 days of screening • Previous exposure to ixmyelocel-T, allogeneic cell therapy, or autologous cell therapy cultured with animal proteins
Other

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• In the opinion of the investigator, the patient is unsuitable for cellular therapy or has a food/drug allergy, surgical or medical condition, clinically significant psychiatric disorder, poor nutritional status, or lab abnormality requiring further medical evaluation that may interfere with the investigational product, interpretation of the trial results, or the patient's ability to complete the trial, or compromise patient's safety

Abbreviations: VAD, ventricular assist device.

Table 3. Ixmyelocel-T Lot Release Criteria

Description	Test Parameter	Lot Release Specification
Viability	%Viable	≥85%
Identity	%CD90+	≥3%
	%CD14+Auto+	≥4%
	%CD45+ plus %CD90+	≥90%
	CD235a+ cell number	≤30 x 10^6
Potency	Metabolic activity	≥0.40 OD per 0.125 x 10^6 cells
Dose	Viable nucleated cell number	40 – 200 x 10^6 cells
Volume	Total liquid volume	5.8 – 8.4 mL
Sterility	In-process product	No growth day of release, and following 7 day culture
	Final product	None; no growth detected following 14 days in culture
	Gram stain	No organisms observed
	Mycoplasma	None; No growth detected following 28 days in culture
	Endotoxin	<100 EU
Animal protein residuals	Total quantity BSA in final product	≤15.0 µg BSA

Abbreviations: BSA, bovine serum albumin; EU, endotoxin units; OD, optical density

Table 4. Primary and Key Secondary Endpoints

Primary endpoint: Total number of all-cause deaths, cardiovascular hospitalizations, and unplanned outpatient and ED visits to treat ADHF over 12 months following administration of study treatment, excluding events considered related to the administration procedure

Key secondary endpoints (efficacy)
• The win ratio over 12 months following administration of study treatment
• Change from baseline in the following parameters at 3, 6, and 12 months after treatment:
  ○ Functional: 6MWT
  ○ Structural: LVEF, LVESV, LVEDV, LV stroke volume, wall motion index
  ○ Symptomatic and quality of life: NYHA classification, MLHFQ total score, EQ-5D summary index

Safety endpoints • Aspiration site assessments; percutaneous catheter site assessments; post catheterization monitoring • AEs, including suspected related immunologic reactions • Immune response results
Exploratory endpoints • MRU data for pharmacoeconomic analyses

Abbreviations: EQ-5D, EuroQol 5D Questionnaire; LVEDV, LV end diastolic volume; LVESV, LV end systolic volume; MHLFQ, Minnesota Living with Heart Failure Questionnaire.

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