

*Trial Designs***Rationale and Design of the SENECA (StEm cell iNjECTION in cAncer survivors) Trial**

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ABSTRACT

Objectives: SENECA (StEm cell iNjEction in cAncer survivors) is a phase I, randomized, double-blind, placebo-controlled study to evaluate the safety and feasibility of delivering allogeneic mesenchymal stromal cells (allo-MSCs) transendocardially in subjects with anthracycline-induced cardiomyopathy (AIC).

Background: AIC is an incurable and often fatal syndrome, with a prognosis worse than that of ischemic or nonischemic cardiomyopathy. Recently, cell therapy with MSCs has emerged as a promising new approach to repair damaged myocardium.

Methods: The study population is 36 cancer survivors with a diagnosis of AIC, left ventricular (LV) ejection fraction $\leq 40\%$, and symptoms of heart failure (NYHA class II-III) on optimally-tolerated medical therapy. Subjects must be clinically free of cancer for at least two years with a $\leq 30\%$ estimated five-year risk of recurrence. The first six subjects participated in an open-label, lead-in phase and received 100 million allo-MSCs; the remaining 30 will be randomized 1:1 to receive allo-MSCs or vehicle via 20 transendocardial injections. Efficacy measures (obtained at baseline, 6 months, and 12 months) include MRI evaluation of LV function, LV volumes, fibrosis, and scar burden; assessment of exercise tolerance (six-minute walk test) and quality of life (Minnesota Living with Heart Failure Questionnaire); clinical outcomes (MACE and cumulative days alive and out of hospital); and biomarkers of heart failure (NT-proBNP).

Conclusions: This is the first clinical trial using direct cardiac injection of cells for the treatment of AIC. If administration of allo-MSCs is found feasible and safe, SENECA will pave the way for larger phase II/III studies with therapeutic efficacy as the primary outcome.

Keywords: cell therapy, anthracycline-induced cardiomyopathy, trial design, mesenchymal stromal cells, allogeneic

INTRODUCTION

Anthracycline-based chemotherapies remain common and effective treatments for breast cancer, lymphomas, leukemias, and sarcomas. Unfortunately, use of anthracyclines is limited by cardiotoxicity, which may manifest as late as 20 years after initial exposure¹. The risk of anthracycline-induced cardiomyopathy (AIC) is primarily related to the lifetime cumulative dose of anthracyclines, but even at low cumulative doses (such as standard-dose therapy for breast cancer), some patients will have evidence of AIC as demonstrated by endomyocardial biopsy or clinically-significant decline in left ventricular (LV) ejection fraction (EF)²⁻⁴. Thus, every exposure to an anthracycline carries some risk of inducing cardiac dysfunction. AIC often is irreversible and carries a prognosis that is worse than that of ischemic or nonischemic cardiomyopathy, with a ~3.5 higher relative risk of dying within five years⁵. Many patients with AIC are relatively young, and ~70% are women⁶. Several strategies have been tested to decrease the incidence of AIC, with little or no success⁷. Other than heart transplant or mechanical support device, current therapies are palliative, and AIC remains an incurable and often fatal disease for which no effective treatment is available.

Given the limited availability of donor hearts and the bleak prognosis of AIC, new treatment strategies are sorely needed; this is an important unmet need in contemporary cardiovascular medicine. Recently, cell-based therapies with bone marrow-derived mesenchymal stromal cells (MSCs) have emerged as a novel approach to regenerate and/or repair damaged myocardium^{8,9}. Since cell therapy appears to be a safe and promising treatment for regenerating/repairing myocardium and improving cardiac function in both ischemic cardiomyopathy⁸⁻¹¹ and dilated nonischemic cardiomyopathy^{12,13}, we postulated that it may be beneficial in AIC as well; in all of these conditions, the underlying cause of heart failure is the loss of viable myocytes with replacement fibrosis. We selected allogeneic MSCs (allo-MSCs) to obviate the risk of disseminating the original malignancy and because mounting evidence supports the potential therapeutic utility of these cells in augmenting LV function both in a preclinical model of AIC¹⁴ and in patients with ischemic or nonischemic cardiomyopathy¹¹⁻¹³. Accordingly, we designed the SENECA trial

(StEm cell iNjEction in cAncer survivors) to test the safety and feasibility of administering allo-MSCs in subjects with AIC.

METHODS

Study Design. SENECA is a phase I, randomized, double-blind, placebo-controlled study to evaluate the safety and feasibility of delivering allo-MSCs by the transendocardial route in subjects with LV dysfunction secondary to AIC (clinicaltrials.gov Identifier: NCT02509156). This first-in-human trial, funded by the National Heart, Lung, and Blood Institute (NHLBI) and sponsored by the Cardiovascular Cell Therapy Research Network (CCTRN), will also collect preliminary evidence for the therapeutic efficacy of allo-MSCs in this population. The primary hypotheses are: (i) that allo-MSCs are well-tolerated (safety) and (ii) that they can be delivered as planned and the outcome measures can be collected (feasibility). The secondary hypothesis is that allo-MSCs promote myocardial repair, thereby reducing scar burden and improving cardiac performance, functional capacity, and quality of life (efficacy). The design of the study was determined in consultation with the Food and Drug Administration, while the drafting and editing of this paper was the sole responsibility of the authors. While the independent safety profiles of both allogeneic MSCs and transendocardial injection are well known, introduction of the study product *for the first time* in subjects with AIC warranted an additional safety assessment prior to initiating a placebo arm. The lead-in evaluation assuaged any concerns of acute complications related to the performance of 20 transendocardial injections of 0.4 ml each throughout the left ventricle in a diffusely fibrotic heart.

Study Population. Inclusion and exclusion criteria are summarized in Tables 1 and 2, respectively. Candidates for enrollment include men and women, aged 18 to 79, who are cancer survivors with a diagnosis of AIC. Subjects must have LV systolic dysfunction ($LVEF \leq 40\%$) and symptoms of heart failure (NYHA class II-III), and must be receiving stable, optimally-tolerated heart failure therapy (e.g., beta blockers, ACE inhibitors/ARBs, and/or aldosterone antagonists), unless contraindicated. Subjects

must be clinically free of cancer (except carcinoma *in situ* and fully resected basal and squamous cell cancer of the skin) for at least two years and must have an estimated five-year risk of recurrence $\leq 30\%$. Ongoing antiestrogen, HER2, or rituximab maintenance are not considered chemotherapy for the purposes of this definition, and therefore are permissible during study participation. Following preliminary review of their medical records to determine eligibility, potential subjects will meet with the research staff to review the protocol, the informed consent document, and possible risks of participation. To exclude heart failure secondary to ischemic cardiomyopathy, available imaging obtained within five years of enrollment will be reviewed. Acceptable imaging for detection of CAD includes a coronary arteriogram, a rest/stress SPECT/PET/CT scan, a stress echocardiogram, a stress MRI, or cardiac computed tomography angiography (CCTA). Any of these tests is acceptable provided there have been no symptoms or evidence of CAD since the test. If none of these tests is available within the past five years, the subject will have one of them performed before entry into the study to confirm absence of obstructive CAD or ischemia.

A total of 36 subjects will be enrolled. The first six subjects participated in an open-label, lead-in phase; all of them received active treatment (100 million allo-MSCs via transendocardial injection). After a 1-month safety review of these first six subjects by an NHLBI-sponsored Data and Safety Monitoring Board (DSMB), approval was granted to enroll the remaining 30 subjects, who will be randomized 1:1 to receive either 100 million allo-MSCs (n=15) or cell-free Buminatone solution (n=15) via transendocardial injections.

Baseline Testing and Randomization. Following informed consent, subjects begin baseline testing. The period from consent to treatment will not exceed 45 days (Figure 1). After successful completion of the baseline evaluation (Table 3), subjects are randomized via an online access database created and maintained by the Data Coordinating Center (DCC).

Cell Manufacturing. Allo-MSCs were manufactured by the Center for Cell and Gene Therapy (CAGT; Baylor College of Medicine, Houston, TX) using donor marrow from a healthy, young female volunteer meeting federal eligibility requirements for donors of human cell and tissue products (including infectious disease testing, risk behavior assessment, and clinical examination)¹⁵. The cell product was grown and expanded using a functionally-closed computer-controlled bioreactor (Terumo Quantum Cell Expansion System). Cells are cryopreserved at the CAGT and transported to each clinical site, via validated liquid nitrogen dry shipper, within one week prior to scheduled injection. Placebo product consists of cell-free Buminate solution. Local cell processors receive and prepare all products for administration. Both study products (allo-MSCs and placebo) are provided to treatment teams in packaging with identical appearance.

Study Product Delivery. Each subject receives 20 transendocardial, electromechanically-guided injections performed with the NOGA MyoStar injection catheter (Biologic Delivery Systems) (Figure 2). The intervention group receives a total of 100 million allo-MSCs in 8 ml of Buminate solution; the placebo group receives 8 ml of cell-free Buminate solution. Electromechanical mapping is used to identify target areas for injection based on viability according to unipolar voltage. Priority for injection is given to areas of relative fibrosis demonstrating intermediate levels of unipolar voltage (4-8 mV); however, areas with unipolar voltage up to 12 mV can be included to treat the entire left ventricle. In addition, injection sites should satisfy the following two criteria: (i) perpendicular position of the catheter to the LV wall and (ii) loop stability <4 mm. After proper placement of the catheter is confirmed, subjects in the treatment group receive transendocardial injections of 0.4 ml of solution (approximately 5 million allo-MSCs per injection). Those in the placebo group receive vehicle (0.4 ml of Buminate solution per injection). All subjects are monitored overnight. After the procedure, a transthoracic 2-D echo without contrast is performed to evaluate subjects for pericardial effusion, and serial blood samples for troponin measurements are collected for 24 hours.

Outcomes. The primary endpoints are safety and feasibility. All adverse events grade 2 and higher, based on the Common Terminology Criteria for Adverse Events (CTCAE), are captured. These events include major adverse cardiac events (MACE) and other significant clinical events (Table 4). The feasibility of preparing and delivering the study product, as well as collecting cardiac MRI variables in subjects with cardiac devices, is assessed.

Efficacy measures of interest are obtained at baseline, 6 months, and 12 months, permitting an assessment of their trajectory over time and whether that trajectory is modified by therapy. MRI evaluations of LV function, structure (including LV volumes), fibrosis, and scar burden provide a comprehensive assessment of myocardial performance and morphology. All MRI analyses are performed in a core laboratory by investigators masked to treatment assignment and other subject data. Functional capacity encompasses exercise tolerance (six-minute walk test)¹⁶ and quality of life assessment (Minnesota Living with Heart Failure Questionnaire). We also assess clinical outcomes (measured by MACE and cumulative days alive and out of the hospital) and biomarkers of heart failure (NT-proBNP).

Statistical methods. The principal analysis will be based on “intention-to-treat”. The reasons for non-treatment of the anticipated small number of subjects who are randomized but do not receive treatment will be tabulated. All analyses will be performed on two cohorts: first, on the 30-subject randomized cohort and then on the total cohort (randomized plus the six open-label therapy subjects). Descriptive statistics for baseline characteristics, known or suspected to be associated with outcomes, will be prepared for the treatment groups, including, but not limited to, demographic characteristics, medical history, physical examination, laboratory data, and quality of life and psychosocial data. Exact testing for categorical variables and Wilcoxon two-sample rank sum test for continuous variables will be used to evaluate the differences in baseline variables between treatment groups. There are two prospectively declared follow-up durations: 6 months and 12 months after injection. For each of these periods, the change in every efficacy measure of interest will be computed. The difference in these changes between the two treatment groups will be described and evaluated using Wilcoxon two-sample procedures, with

regression modeling to adjust for a small number of treatment confounders. No adjustment for multiplicity is anticipated for this feasibility trial.

Follow-up visits and surveillance. In both the lead-in and the main trial, subjects are followed for 12 months for safety and efficacy. Follow-up initiates on the day of injection and subsequent visits occur at day 1, 1 week, 1 month, 6 months (primary endpoint assessment), and 12 months (primary endpoint assessment). All available standard-of-care medical records for cancer surveillance are reviewed for cancer recurrence at 12 months. A telephone contact takes place at 24 months to assess the subject's current medications, as well as morbidity and mortality.

Safety monitoring. The DCC employs a comprehensive and data driven safety monitoring program that provides ongoing capture and analysis of safety data, and generates timely notifications and reports of safety issues to appropriate study personnel (for local institutional review board reporting) to the NHLBI DSMB and to the Food and Drug Administration (FDA). All SENECA subjects are closely monitored for adverse events with transmission of information to these relevant oversight groups. The DSMB meets at least twice annually to review performance of participating centers, assess recruitment, evaluate study progress, and report recommendations to the NHLBI. The DCC monitors regulatory and safety compliance at each clinical center and conducts regular visits to each site to ensure protocol adherence.

Biorepository. A central biorepository is utilized. The goals are to provide: i) storage of critical biomaterials (i.e., subject peripheral blood and donor allo-MSC product), ii) long-term integrity (up to 10 years) of these biospecimens and products, and iii) management of immunologic, immunohistochemical, cellular, and molecular analyses of donor allo-MSC product and subject serum samples, as well as phenotypic and functional analyses of cells and plasma samples. The overall aim of this central biorepository is to gain insights into the diagnostic accuracy of disease progression and prognostic information into successful intervention.

DISCUSSION

SENECA is the first clinical trial to utilize direct cardiac injection of cells for the treatment of AIC – an incurable and often fatal syndrome that disproportionately affects women and young adults, and for which no effective treatment currently exists^{5,7}. This first-in-human study has the potential to herald a novel approach to the management of AIC.

Despite attempts at improving therapy, the prognosis of AIC is grim, worse than that of ischemic or idiopathic cardiomyopathy^{5,7}. Anthracyclines induce cell death and fibrosis via free radical production, DNA damage, and altered intracellular signaling¹⁷. The biological hypothesis underlying SENECA is that allo-MSCs may promote replacement of lost cardiomyocytes, repair of injured cardiomyocytes, decrease in interstitial myocardial fibrosis, and/or reduction of chronic inflammation, all of which may promote restoration of cardiac tissue and function. As noted earlier, the primary endpoints are safety and feasibility. If administration of allo-MSCs is found to be feasible and safe, SENECA will pave the way for larger phase II/III studies whose primary endpoint will be therapeutic efficacy. Clearly, the results of SENECA (e.g., changes in LV function and functional status) will be crucial to inform the design of such studies and ensure that they are adequately powered.

Preclinical studies of cell therapy in AIC. The concept that cell therapy is beneficial in AIC is supported by preclinical observations. De Angelis *et al.* induced AIC in rats with a three-week doxorubicin treatment; subsequent treatment with syngeneic c-kit⁺ cardiac stem cells resulted in a 66% decrease in mortality at 6 weeks and a significant improvement in multiple parameters of LV function compared with control rats treated with vehicle¹⁸.

Psaltis *et al.* examined the safety and utility of allo-MSCs delivered transendocardially in a sheep model of AIC¹⁴. In this study, allo-MSCs were prepared from ovine bone marrow by immunoselection using the tissue nonspecific alkaline phosphatase, or STRO-3, monoclonal antibody. Fifteen sheep with AIC were assigned to catheter-based, transendocardial injections of ovine allo-MSCs (n=7) or placebo (n=8) under

electromechanical mapping guidance. Ten weeks after the first exposure to doxorubicin, animals received 20 injections of placebo or allo-MSCs distributed throughout the left ventricle. At 8 weeks after treatment, LV end-diastolic volume (LVEDV) increased similarly in both cohorts; however, allo-MSC therapy attenuated the increase in LV end-systolic volume (LVESV) compared with the control group ($P < 0.01$). LV ejection fraction (LVEF) increased from $37.3 \pm 2.8\%$ before cell therapy to $39.2 \pm 1.4\%$ at 8 weeks after cell therapy ($P = \text{NS}$) in treated sheep, whereas in control animals it decreased from $38.8 \pm 4.4\%$ to $32.5 \pm 4.9\%$ ($P < 0.05$) at corresponding time points. Histological analysis showed that allo-MSC therapy was associated with less fibrosis than in the control group and increased density of karyokinetic cardiomyocytes and arterioles ($P < 0.05$ for each), although the engraftment of cells after transplantation was modest. This study, conducted in a large animal model of AIC, provides evidence for the therapeutic utility of allo-MSCs in the treatment of AIC, and thus supports the rationale for SENECA.

Rationale for performing SENECA in the CCTRN. Because the prevalence of AIC is lower than that of ischemic or idiopathic cardiomyopathy,^{5,19-21} conducting a phase I trial of AIC in one center would be difficult, if not impossible. On the other hand, by virtue of its multicenter structure, the CCTRN is an ideal resource for such a study. The lead-in phase (with six subjects) began on September 22, 2016 and its 1-month follow-up ended on January 24, 2017. The main trial began on March 2, 2017; based on a conservative projection, we expect that the infrastructure of the CCTRN (consisting of seven clinical centers) will enable us to enroll 30 subjects within 12 months, highlighting the advantages of a network infrastructure for clinical trials of cell therapy. .

SENECA is an example of the benefits that accrue from an interdisciplinary cooperation. Indeed, this trial requires a close partnership among cardiologists, oncologists, and primary care physicians in addressing a common problem that represents a major cause of morbidity and mortality in this specific patient population. Several aspects of SENECA were designed in collaboration with oncologists and cancer researchers (Kathy Miller, Indiana University; Tan Ince, University of Miami; Carrie Lenneman, University of Louisville).

Selection of endpoints. Because the effects of allo-MSCs in AIC are unknown, SENECA is designed as a phase I, hypothesis-generating study. We will assess not only changes in LV volumes, LV function, and myocardial structure (e.g., fibrosis), but also changes in functional capacity (measured by the six-minute walk test and the MLHF questionnaire), clinical outcomes (measured by MACE and cumulative days alive and out of the hospital), and biomarkers of heart failure (NT-proBNP). This broad set of endpoints will ensure that beneficial actions of allo-MSCs are not overlooked and will provide information essential for selecting the most appropriate endpoint and sample size for a subsequent phase II trial.²² As outlined in Table 4, cMRI will be used to evaluate myocardial function and structure. cMRI was selected over other imaging modalities, such as cardiac CT and echocardiography, because it enables accurate, high-resolution assessment of myocardial injury (e.g., fibrosis, inflammation, edema)²³⁻²⁵, is considered to be more reproducible for measuring LV function and volumes^{26,27}, and does not require additional radiation exposure. In addition, cMRI enables assessment of myocardial strain, which may have greater sensitivity than LVEF in predicting clinical outcome²⁸, particularly in chemotherapy-induced cardiomyopathy²⁹. Accordingly, use of cMRI may allow for an improved assessment of the benefits of cell therapy. While not all subjects will require a cardiac device, it is anticipated that 60-65% of participants in the study population will have one. The presence of most pacemakers or implanted defibrillator devices is not a contraindication to MRI scanning in SENECA^{30,31} (Table 2).

Safety of allo-MSCs in cancer patients. The experience obtained thus far with administration of adult progenitor cells for heart disease indicates that this therapy is safe; in particular, there has been no evidence of tumor formation.³² Nevertheless, based on evidence from cell lines, case reports, and clinical studies, concerns have been raised that some cell-based therapies, particularly those involving cells that augment angiogenesis and repress apoptosis, may stimulate growth of active, high-grade tumors³³⁻³⁸; in contrast, cancer in remission does not appear to be activated by cell therapy³⁹. To address such concerns, subjects enrolled in SENECA must have firmly established cancer remission (i.e., be in remission for at least two years) and low risk of recurrence (i.e., estimated five-year risk of recurrence $\leq 30\%$) to be

eligible for the study. Clearly, the outcome of this trial will be of utmost importance in elucidating whether administration of MSCs is safe in this population.

Rationale for using *allo*-MSCs. The choice of MSCs is motivated by the fact that this is a particularly promising cell type for cardiac regenerative therapy, due to availability, immunologic properties, and record of safety and efficacy⁴⁰⁻⁴². Studies in rodent and swine models of myocardial infarction have shown that MSC administration results in improved recovery of LV function after MI, neo-angiogenesis at the infarct site, and decreased collagen deposition in the scar region^{43,44}. Differentiation of MSCs into mature cardiomyocytes has not been observed^{43,44}, although MSC-derived cells expressing contractile and sarcomeric proteins but lacking true sarcomeric functional organization have been reported^{45,46}. A potentially important mechanism of action of MSCs is stimulation of endogenous cardiac progenitor cells⁴⁷. Complementary mechanisms that have been shown to play key, perhaps dominant, roles in the salutary cardiac effects of MSCs involve the secretion of paracrine factors^{41,48}. With respect to AIC in particular, recent work has shown that the cell-free secretome produced by human bone marrow- or iPSC-derived MSCs as well as by human MSCs cultured from amniotic fluid is sufficient to attenuate anthracycline toxicity in cardiomyocytes *in vitro* and AIC *in vivo*^{49,50}.

MSCs are thought to be ideal candidate cells for allogeneic transplantation because they show minimal expression of major histocompatibility complex (MHC) class II antigens and intercellular adhesion molecules (ICAM), and they lack B-7 co-stimulatory molecules necessary to trigger T-cell-mediated immune response^{51,52}. In addition, they exert anti-proliferative and anti-inflammatory effects, exhibit immunosuppressive properties, and suppress proliferation of T cells activated by allogeneic cells or mitogens⁵³. In this regard, human MSCs fail to induce proliferation of allogeneic lymphocytes *in vitro*⁵⁴. Finally, recent trials suggest safety and efficacy of *allo*-MSCs in patients with nonischemic dilated cardiomyopathy¹¹⁻¹³. Taken together, these considerations support the concept that MSCs represent a promising cell type for allogeneic cell therapy⁴¹.

The rationale for administering allo-MSCs (rather than autologous MSCs) in SENECA is multifarious. Use of allogeneic cells obviates any concern that autologous cells may lead to dissemination of the original malignancy for which patients were treated with anthracyclines. Clearly, recurrence or dissemination of the original neoplasm would be a catastrophic outcome that prohibits use of autologous cell therapy. Administration of allogeneic cells also avoids the challenge of harvesting potentially compromised progenitor cells from a subject with severe co-morbidities or advanced age, both of which are known to alter the phenotype of autologous MSCs⁵⁵. Allo-MSCs have been used in several recent FDA-approved trials with encouraging results^{11,13}, and one trial suggests that they may be more effective than autologous MSCs¹². The cumulative experience from these studies has demonstrated not only an outstanding safety margin but also a considerable potential for allo-MSCs to exert beneficial effects in heart failure patients¹¹⁻¹³.

Rationale for using the dosage of 100 million allo-MSCs. Our dosing strategy is based upon three major considerations: i) safety data, ii) cell concentration, and iii) total number of injections. By dividing 100 million MSCs into 20 injections of 0.4 ml each, the treatment regimen balances the desire for broad distribution of cells throughout the ventricle with the maintenance of an optimally low concentration of cells (12.5 million/ml). The experience accumulated with clinical trials of MSCs^{11-13,56,57} provides substantial evidence of safety with doses up to 200 million MSCs, as well as preliminary evidence of efficacy. In SENECA, subjects receive a total of 100 million allo-MSCs, divided into 20 injections of 5 million cells each. A dose of 100 million MSCs, delivered transendocardially, has been used in several clinical trials, such as POSEIDON¹¹, TAC-HFT⁵⁶, POSEIDON-DCM¹², and a study by Perin *et al.*¹³, and has proven to be safe. The rationale for selecting 100 million cells, rather than a greater dose, is that in the POSEIDON trial¹¹, there was an inverse dose-response relationship, leading the authors to speculate that higher doses may be less efficacious due to deleterious effects on cell retention, survival, or performance. The 100-million dose appeared to give an efficacy signal in POSEIDON-DCM¹².

Data from preclinical investigations also are consistent with an inverse dose-response relationship. In an ovine model of acute MI treated with transendocardial injection of 25, 75, 225, or 450 x 10⁶ STRO-3 MSCs, Hamamoto *et al.* demonstrated that, compared with control animals, low doses (25 and 75 x 10⁶ cells) of MSCs significantly attenuated the increase in LVEDV and LVESV; the 225 x 10⁶ dose improved only the LVESV over control, whereas the 450 x 10⁶ dose did not produce any salutary remodeling effect relative to controls⁵⁸. In addition, infarct expansion was attenuated only in the 25, 75, and to some extent the 225 x 10⁶ groups, although LVEF improved at all cell doses. CD31 and smooth muscle actin (SMA) immunohistochemical staining demonstrated increased vascular density in the border zone only at the two lower cell doses⁵⁸. Another ovine study used a protocol and allo-MSC doses similar to the above study and arrived at similar conclusions: the LVEDV increase was significantly attenuated only in the 25 and 75 x 10⁶ groups compared with controls, although LVEF improved with all doses of allo-MSCs in comparison with the control animals (P<0.05)⁵⁹. In summary, both preclinical and clinical data suggest that the beneficial effects of transendocardially administered MSCs may decrease as the dose approaches 200-225 x 10⁶.

Rationale for using the transendocardial route of administration. In AIC patients, myocardial fibrosis is diffuse, with a unique cardiac MRI profile^{20,60}. Injections of study product in SENECA targets areas of diffuse fibrosis using the NOGA technology, which can assess in real time and 3-D space underlying myocardial viability based on unipolar voltage. The choice of transendocardial delivery is based on both preclinical and clinical data. The aforementioned study by Psaltis *et al.* in an ovine model demonstrated that MSC therapy delivered by the NOGA device is safe and effective in the treatment of doxorubicin-induced cardiomyopathy¹⁴. Several studies in porcine models of ischemic cardiomyopathy have shown therapeutic efficacy of transendocardially delivered MSCs^{43,61-63}. Moreover, there is substantial clinical experience with the transendocardial delivery of autologous bone marrow-derived mononuclear cells as well as autologous MSCs and allo-MSCs in the setting of chronic LV dysfunction. Many studies^{11,13,56,57,64-67} confirm the safety of NOGA-mediated injections in patients with cardiomyopathy,

including studies that have used up to 20 transendocardial injections^{11,14,67}. In addition, lower cell concentrations have been reported to be associated with greater efficacy^{13,59}. Based on these considerations, we have chosen a volume of 0.4 ml, which is in keeping with previous trials demonstrating safety of volumes of 0.2-0.5 ml (FOCUS, TAC-HFT, RENEW, POSEIDON DCM, etc.)^{11,56,64,65,68,69}. On the other hand, intracoronary delivery of MSCs is thought to be potentially hazardous because of the possibility of producing microvascular obstruction with resultant dose-dependent myocardial damage, as suggested by preclinical studies^{70,71}. In addition, no published clinical trial supports the efficacy of intracoronary MSC delivery, in contrast to copious literature supporting the efficacy of transendocardial MSC delivery, as discussed above. Finally, unlike in ischemic cardiomyopathy in which cell injections are provided in a relatively small area of myocardium surrounding a scar, the diffuse nature of the disease requires that in the SENECA protocol the injections be dispersed through the entire left ventricle.

In summary, SENECA is the first clinical trial using direct cardiac injection of a cell product for the treatment of anthracycline-induced cardiomyopathy. This phase I trial will evaluate the safety and feasibility of delivering allo-MSCs transendocardially in a patient population that has limited therapeutic options and a grim prognosis. If the results are encouraging, SENECA will inform future larger studies of this new therapeutic modality.

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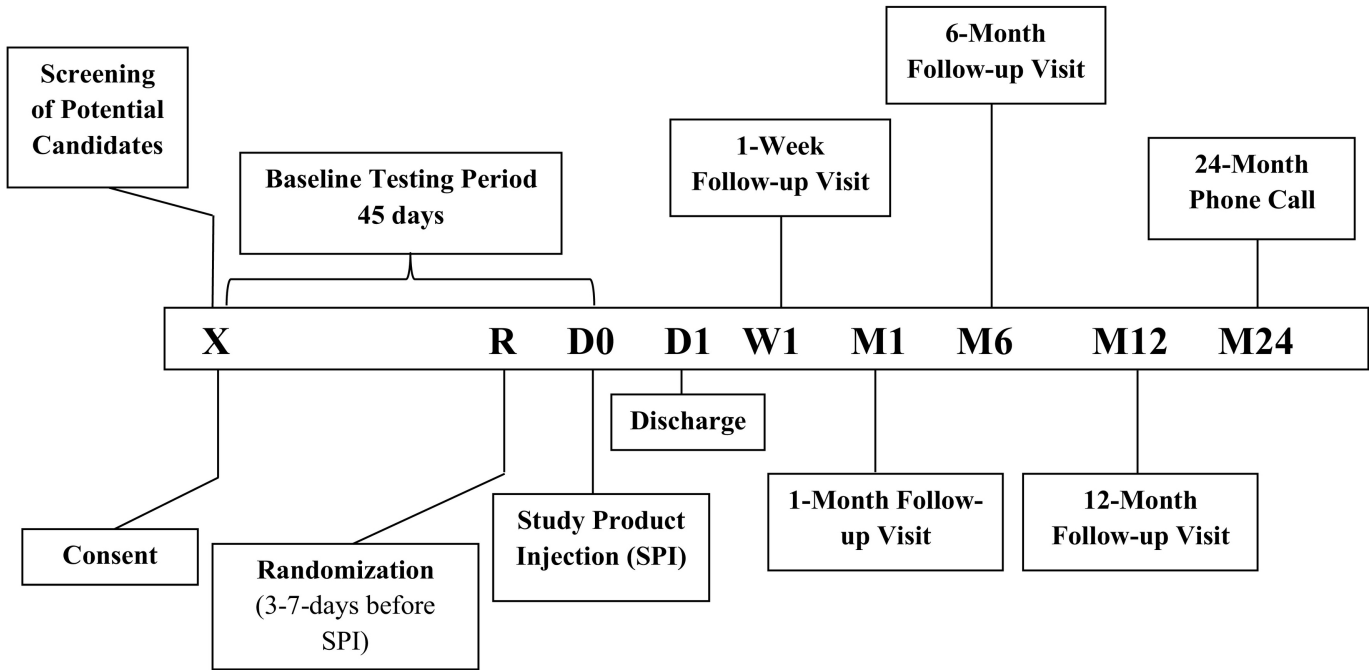


Figure 1

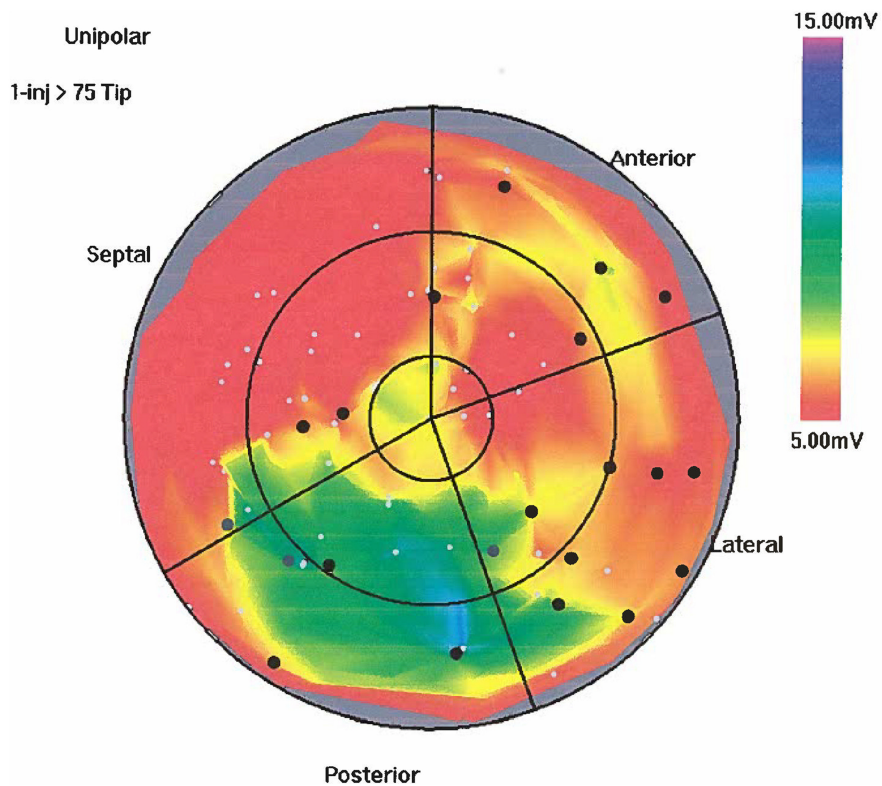


Figure 2