THE LANCET

Supplementary webappendix

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Supplement to: Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet* 2011; published online Nov 14. DOI:10.1016/S0140-6736(11)61590-0.

Online Supplement

Methods

Study stopping rules. The trial was planned and conducted in compliance with the FDA regulations outlined in document 21 CFR 312, subpart D, regarding the stopping of a clinical study and procedures to be followed in the case of severe adverse events related to the administration of the test product (in this case, CSCs). In particular, the rules specified that the study would be stopped upon occurrence of one of the following:

1. Development of cardiac tumor at any time after product of administration.
2. Development of one systemic infection within 2 weeks of product administration.
3. Occurrence of two myocardial infections within 30 days of product administration.
4. Occurrence of two sudden deaths within 30 days of product administration.

Telomere length. Quantitative measurements of telomere length were made by Q-FISH and confocal microscopy or by flow-FISH [1, 2, 3]. Cells were fixed, digested with pepsin, and hybridized with a fluorescein isothiocyanate (FITC)-labeled (C3TA2) [3] peptide nucleic acid (PNA) probe. Total fluorescence of the FITC-PNA probe, which corresponded to the content of telomeric sequences per nucleus, was determined by confocal microscopy. Flow-FISH measurements were performed on a FACSCanto instrument (Becton Dickinson) and the cells were gated at G0/G1 based on DNA content.

Telomerase activity. The catalytic activity of telomerase was assessed by quantitative PCR. CSC extracts were incubated in a solution containing reverse transcriptase reaction mix and Taq polymerase (TRAPEZE RT Telomerase Detection kit, Chemicon). HeLa cells were used as positive control and serial dilutions of control template TSR8 were employed for quantification [2, 3].

Population doubling time (PDT) and cellular senescence. CSCs were plated at low-density and the number of cells was determined daily. PDT was computed by linear regression of log2 values of cell number. To determine the fraction of cells that reached replicative senescence and irreversible growth arrest, cultures were stained for the senescence-associated protein p16INK4a [1, 2, 3].

cMR

Image Acquisition: cMR was performed on a 1.5 T scanner (Espree, Siemens Healthcare, Erlangen, Germany) using an 8-channel body coil with ECG gating and breath-hold acquisitions. Black blood imaging and steady-state free cine images in precession (SSFP) was performed for overall anatomic survey and infarct sizing, respectively. Additional parameters included the following: FOV 29-38cm, matrix 192 x 108, IR: 250-350 ms. Short axis and multiple long axis views of delayed myocardial enhancement imaging was acquired 15-25 minutes following intravenous gadolinium. Post-processing was done using the QMass 7.2 software package (Medis; Leiden, The Netherlands).

Infarct Sizing: Infarct sizing was performed using manual delineation with region of interest (ROI) construction around the areas of myocardium showing delayed gadolinium enhancement. Sizing was performed by two independent readers blinded to patient name and time and date of study in randomized fashion. Correlative analysis using linear regression models were performed to see if correlation between readers was noted. Slice-to-slice comparison showed an R value of 0.877 ($p<0.001$). Additionally, Bland Altman plots were constructed which yielded no significant bias toward higher or lower value assessments by either reader.

Wall Thickening: Wall thickening fraction was estimated by establishing point-to-point correspondences perpendicular to the endocardial-epicardial interface using a Laplace method. Wall thickening fraction was calculated by measuring the difference between mean end-systolic wall thickness and mean end-diastolic wall thickness, divided by mean end-diastolic wall thickness, multiplied by 100. Values are reported as an average of all segments within the standard 17-segment model.

Results

Evidence for Previous Transmural Infarction. In most patients, there was evidence that the previous myocardial infarction was transmural. Specifically, in 12 of the 16 patients treated with CSCs and 4 of the 7 patients in the control arm, there was electrocardiographic (pathologic Q-waves) and/or cMR evidence of transmural infarction. In the remaining 4 patients in the treatment arm, cMR could not be performed because of contraindications (presence of ICD, renal failure, etc.), and therefore we could not use this "gold standard" for establishing the presence of a transmural infarct. In 3 of these 4 CSC-treated patients and in 1 of the 3 control patients that did not have ECG and/or cMR evidence of transmural infarction, there were both akinetic segments on the echocardiogram and fixed perfusion defects by sestamibi SPECT imaging in the region perfused by the infarct-related artery; this association is generally considered indicative of a transmural infarct. Thus, there was evidence of a previous transmural MI in 20 of the 23 patients analyzed (15/16 in the treatment arm and 5/7 in the control arm).

Assessment of Safety and Feasibility.

Safety endpoints were assessed during the first 24 h after CSC infusion and at each follow-up visit. After the CSC infusion, patients were monitored overnight to evaluate any immediate adverse effects (e.g., hypersensitivity to the product, coronary embolization leading to MI, arrhythmias, etc.). Then, patients were followed at 1, 2, and 4 weeks. Within that time frame, serial echocardiograms, serological tests, ECGs, Holter monitors, and physical examinations were performed to assess any short-term adverse effects of CSCs (e.g., organ dysfunction, tumorigenesis, arrhythmias, recurrent MI, infections, etc.). At 4, 8, 12, and 24 months after CSC infusion, serial echocardiograms, magnetic resonance imaging, serological tests, ECGs, and physical examinations were performed to detect any long-term adverse effects (similar to the short-term adverse effects).

Feasibility was assessed by determining whether the procedures required to administer CSCs could be carried out properly and according to protocol. For example, we determined whether the right atrial appendage could be harvested, frozen, shipped to Boston, and then utilized for CSC isolation and expansion without problems. We also determined the efficiency of the procedures used for isolation and expansion of CSCs. Our results demonstrate that harvesting and preparation of right atrial appendages are quite feasible, and that CSCs can be isolated in adequate numbers from virtually all atrial appendages harvested (the only patient in whom we were unable to isolate and expand stem cells had cardiac amyloidosis). In addition, we determined whether it is feasible to infuse CSCs intracoronarily using our protocol, without technical problems or adverse effects. Our experience indicates that this is eminently feasible. Finally, our study sought to determine whether it is feasible to isolate stem cells, expand them, transport them to a distant location (from Boston to Louisville), and infuse them into patients without loss of sterility. Our results demonstrate that all of these manipulations can be performed without loss of sterility, viability, or c-kit positivity. Our data also demonstrate that it is feasible to obtain relatively pure populations of CSCs, in which almost 90% of the cells are c-kit positive and almost all of the cells are negative for lineage markers and have consistently high telomerase activity, relatively long telomeres, and a significant growth reserve as assessed by the population doubling time.

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Figure Legends

Supplemental Figure 1. Trial design illustrating the timing of interventions and diagnostic tests utilized to measure trial outcomes. Patients were screened at two time points: during the 2 weeks preceding CABG surgery (initial eligibility screening and enrollment), and at 4 ± 1 month after CABG surgery (final enrollment). Based on treatment allocation, patients underwent interventions and follow-up evaluations according to one of the two protocols shown (CSC-treated arm or control arm). Both groups are assessed at 4, 12, and 24 months after baseline evaluation (baseline was the time of final enrollment, 4 ± 1 months after CABG).

Supplemental Figure 2. CSCs prior to intracoronary delivery. **A,** Confocal images illustrating the localization of c-kit (green) in CSCs of each of the 16 patients. **B,** Telomeres in CSC nuclei (red dots) are identified by Q-FISH and flow-FISH. R cells with long telomeres, 48 kbp, and S cells with short telomeres, 7 kbp, were used to compute absolute values. By flow-FISH, the histograms represent the intensity of PNA probe binding in gated CSCs (red) and control cells (green). **C,** FACS analysis of c-kit expression and lineage markers of cardiac cells in the 16 patients. The number in each panel reflects the patient's code.

Supplemental Figure 3. CSC senescence. Expression of $p16^{INK4a}$ (magenta, arrows) in CSCs from each patient. The number in each panel reflects the patient's code.

Supplemental Fig 4. LV wall thickening fraction, measured by cMR, before and 4 months after CSC infusion in treated patients. Data are means ± SEM.

Supplemental Fig 5. Infarct score index measured by cMR at 4 and 12 months after baseline in treated patients (baseline was 4 ± 1 month after surgical revascularization). Data are means ± SEM.

Supplementary Table 1. Inclusion/Exclusion Criteria

Inclusion Criteria
Left ventricular ejection fraction ≤ 40%
A history of Q-wave MI with a residual akinetic and nonviable scar (as evidenced by a low-dose dobutamine stress echocardiogram and/or a thallium redistribution nuclear study for viability and/or an electrocardiogram and/or perfusion cardiac MRI and/or rest images on sestamibi SPECT imaging)
Patient scheduled for surgical revascularization within 2 weeks of the initial screening
Exclusion Criteria
Age > 75 years old
Cardiogenic shock
Severe co-morbidities (e.g., renal failure, liver failure, life expectancy <12 months)
Mini-CABG procedures
Pregnant/nursing women or women of child bearing age (menstrual period in the last 12 months)
Diabetics with HbA1c > 8.5%
Inability to provide informed consent
Patients will be excluded if they exhibit loss of mental competency after CABG
AST, ALT, alkaline phosphatase, and serum creatinine more than 3 times the upper limit of normal.
Absolute neutrophil count (ANC) < 2000 or total WBC count is more than two times the upper limit of normal
Hemoglobin <9 g/dl and hematocrit <30%
Ventricular tachycardia during the 90 days prior to the time when CSC administration (Prior ICD placement not exclusionary)
Patients with a history of hepatitis B, hepatitis C, and HIV

Supplemental Table 2. Summary of Stem Cell Trials in Subacute/Chronic Ischemic Heart Failure

Type of trial	Author	Age of Infarct	Treated/ Control	Randomized	Change in EF (%) (Controls)	Change in EF (%) (Treated)	Duration of follow-up	Assessment
Epicardial Injection								
CABG + BMC	Stamm et al.[4]	<3 months	6/no control	N	N/A	12.7	3-10 months	Echo
CABG + BMC	Zhao et al.[5]	~18 months	18/18	Y	4	13	6 months	Echo
CABG + BMC	Stamm et al.[6]	2 weeks to 3 yrs	20/20	Y	3.7	9.7	6 months	Echo
CABG + BMC	Ahmadi et al.[7]	<3 months	18/9	N	5.2	3.7	14 months	Echo
CABG + BMC	Patel et al.[8]	Not available	10/10	Y	6.5	16.6	6 months	Echo
Intracoronary Infusion								
BMC or CPC	Assmus et al.[9]	81 months	28/24/23*	Y	-1.2	2.9/-0.4	3 months	LVgram
BMC	Strauer et al.[10]	27 months	18/18	N	-1	7	6 months	LVgram
BMC	Strauer et al.[11]	~ 8.5 years	184/168	N	-3.5	6.2	60 months	LVgram
CABG +BMC	Hu et al.[12]	>3 months	31/29	Y	5.7	10.6	6 months	MRI
Transendocardial Injection								
BMC	Perin et al.[13]	>3 months	14/7	N	-4.15	5.5	2 months	Echo
BMC	Perin et al.[14]	>3 months	20/10	Y	4.8/0.9	3.5/4.5**	6 months	SPECT/ LVgram
BMC or MSC	Williams et al.[15]	4 months to 11 yrs	8/no	N	N/A	~3**	12 months	MRI

* BMC/CPC/Control

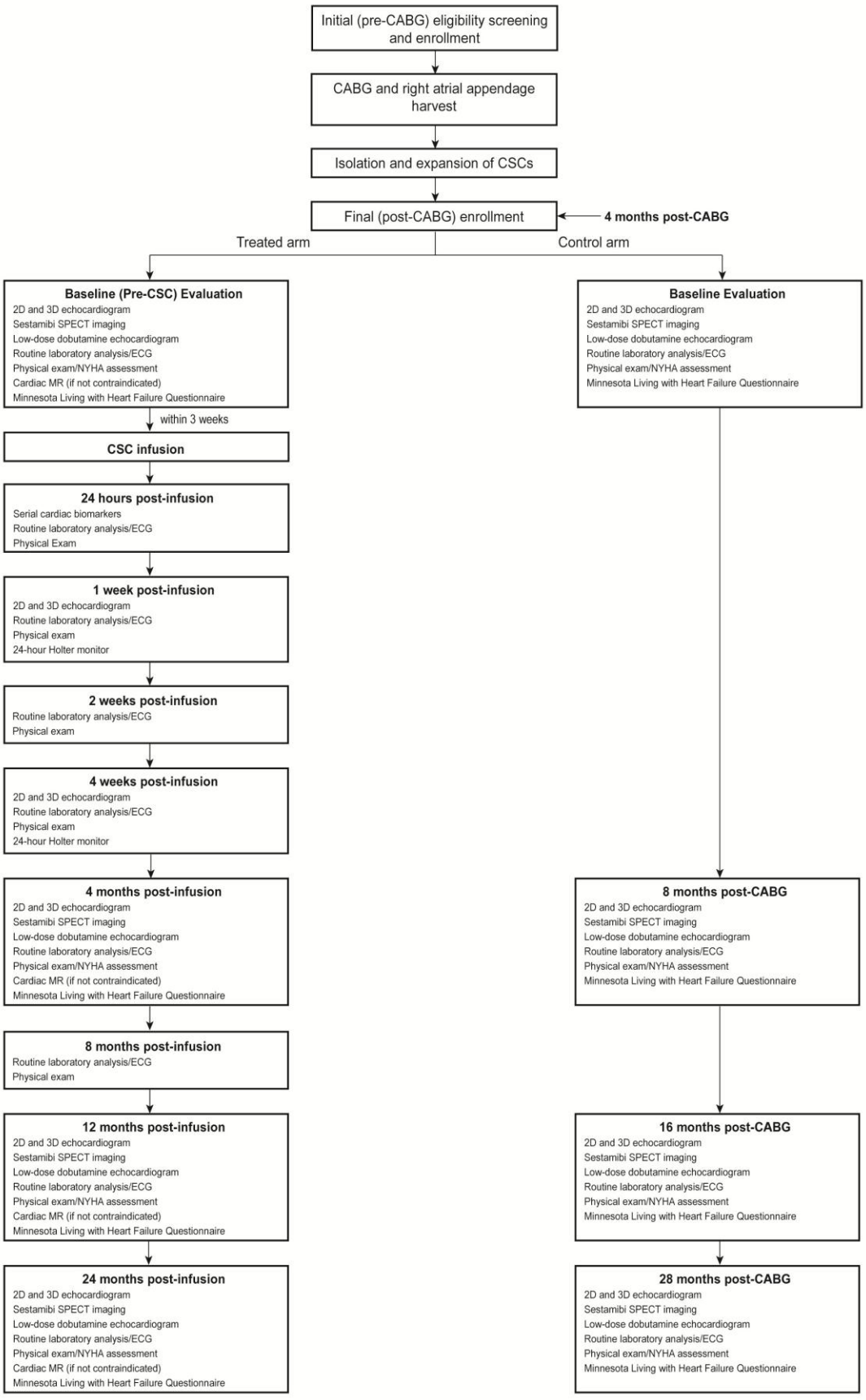
** Not statistically significant when compared to control group

EF	Ejection fraction
CABG	Coronary artery bypass graft surgery
BMC	Bone marrow-derived cells
CPC	Circulating progenitor cells
MSC	Mesenchymal stem cells
SPECT	Single-photon emission computed tomography
LVgram	Left ventriculogram

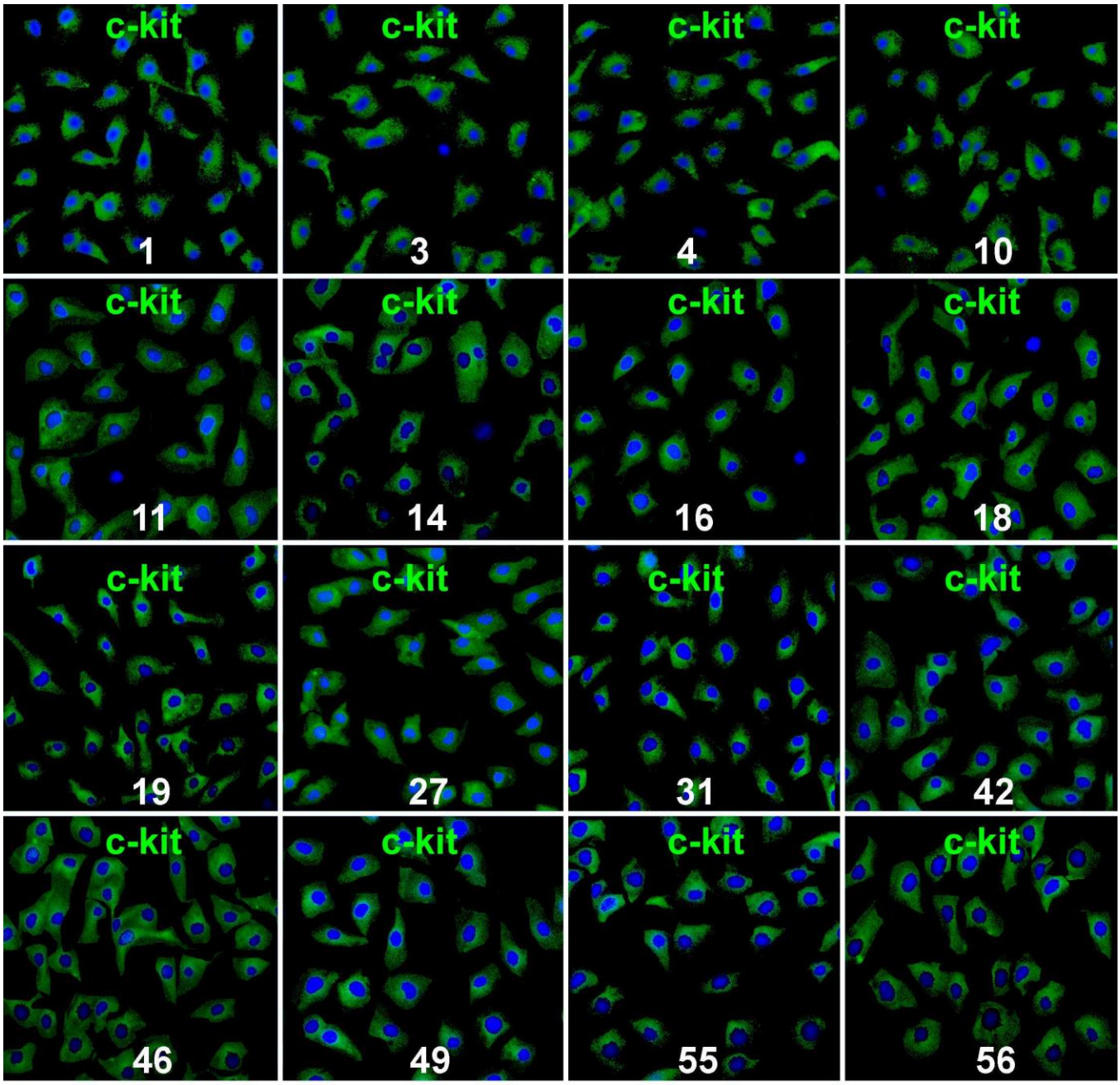
Supplemental Table 3. Protocol Amendments

Date	Amendment	Reason
12/16/2009	Change in exclusion criteria to allow patients with DM with Hgb A1C < 8.5 to be enrolled in the study.	A significant volume of patients with ischemic heart failure are diabetic. It was important to include these patients in the trial while requiring a reasonable level of blood glucose control.
12/16/2009	Adoption of block randomization schema for enrollment which was initiated after enrollment of 9 consecutive treated followed by 4 consecutive controls.	The rationale for block randomization was to attempt to correct the imbalance between the numbers of treated and control patients.
7/29/2010	Number allotted to initial enrollment increased from 60 to 100.	The number of patients excluded secondary to improvement in EF at 4-months post-CABG dictated the need for an increase in the number of patients undergoing initial eligibility screening.
10/11/2010	Removal of exclusion criterion of history of myocardial infarction within the previous week.	Initial concerns for the difficulty of isolating CSCs under the extreme conditions surrounding an acute MI were the reason for enforcing this criterion. Improved culture techniques made this requirement obsolete.

Supplementary Figure 1



Supplemental Figure 2A



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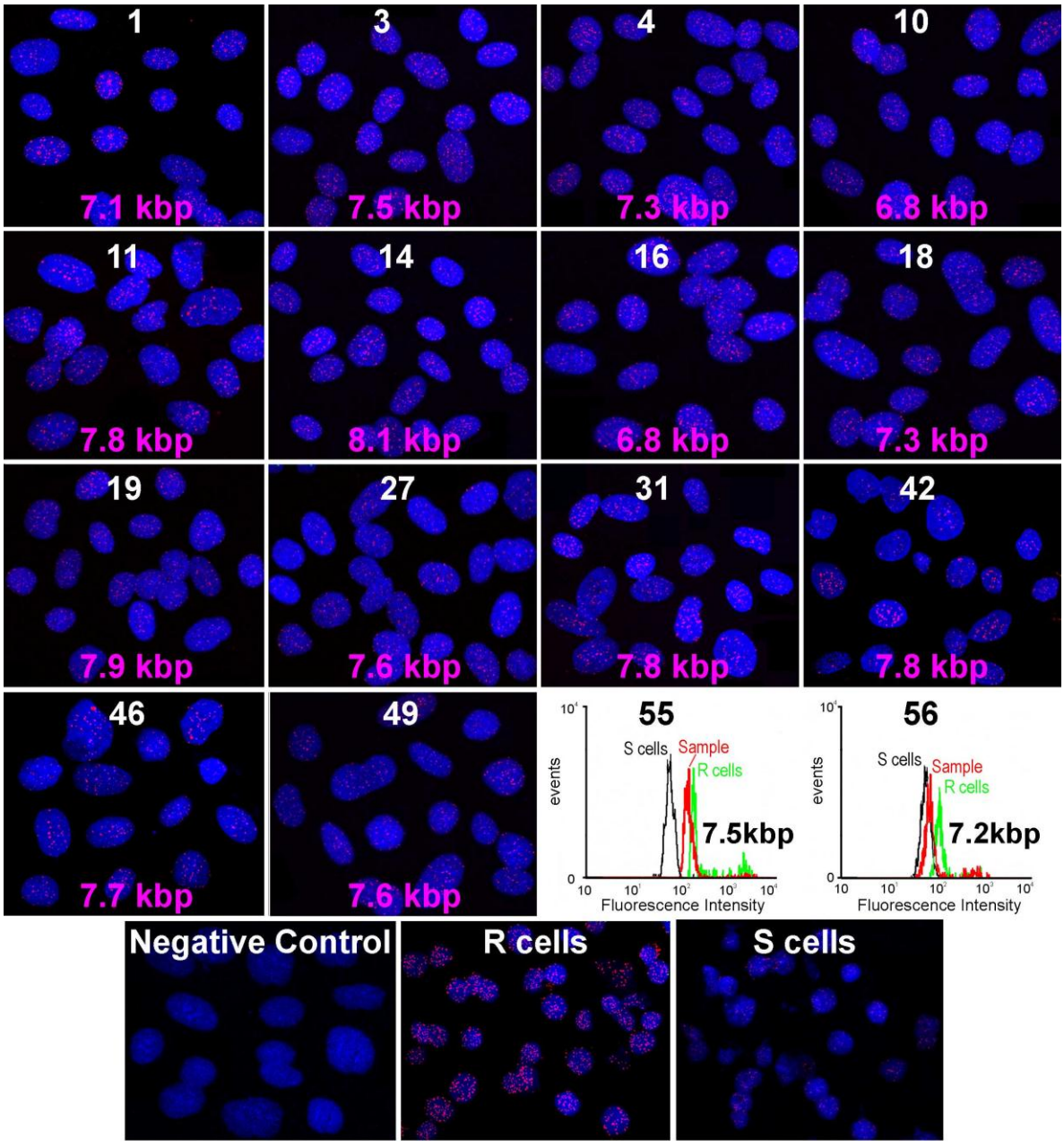

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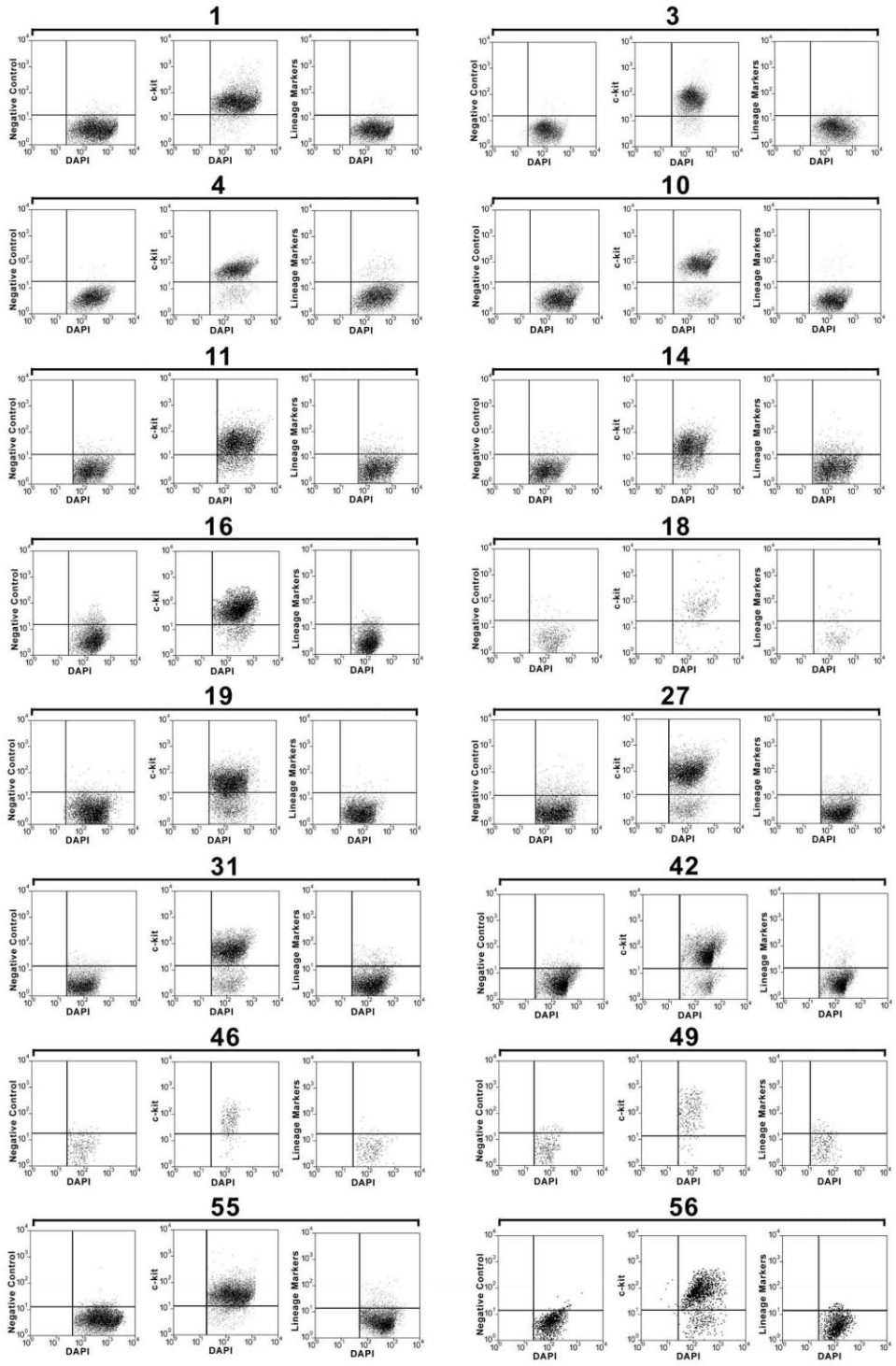

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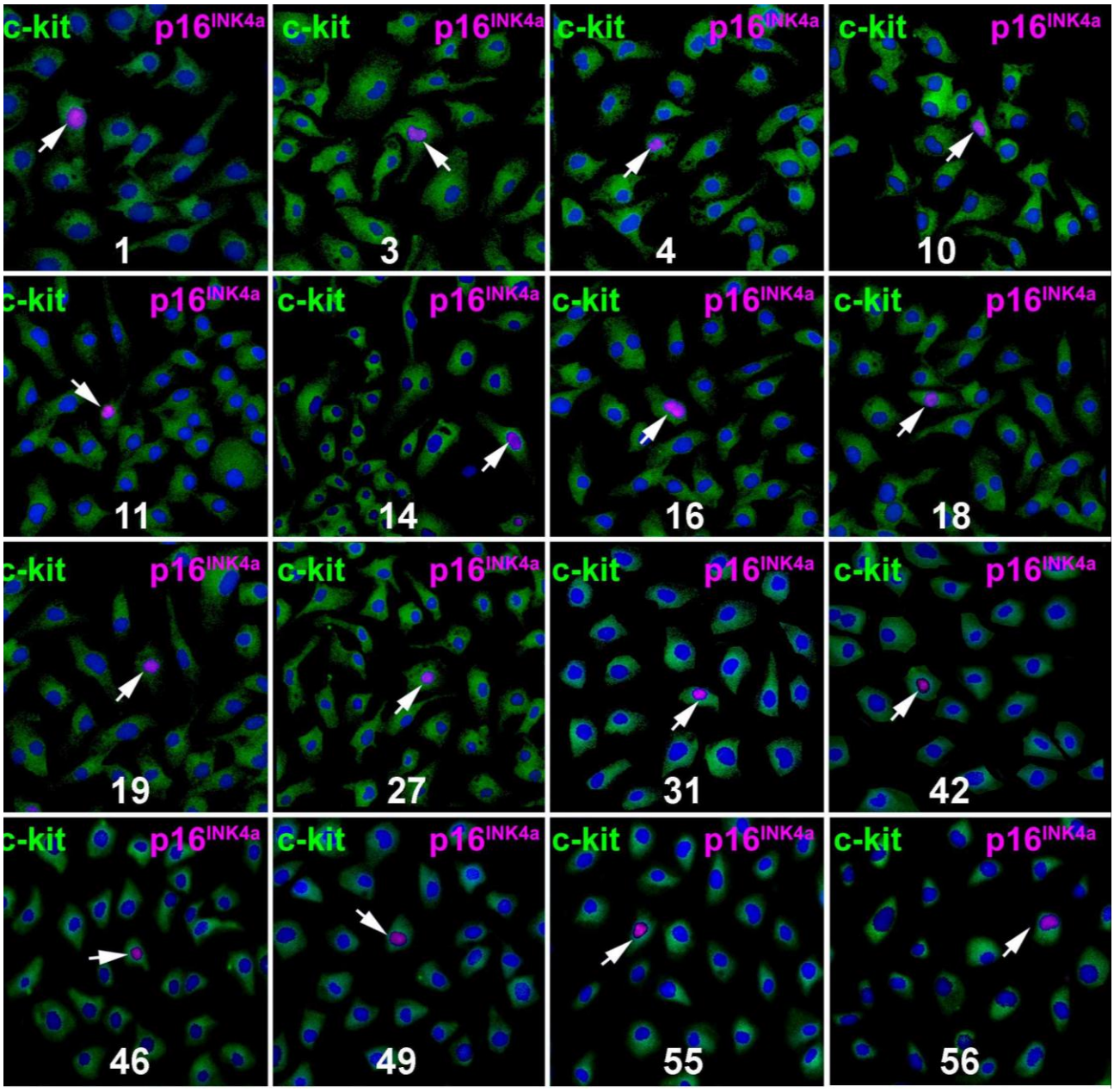
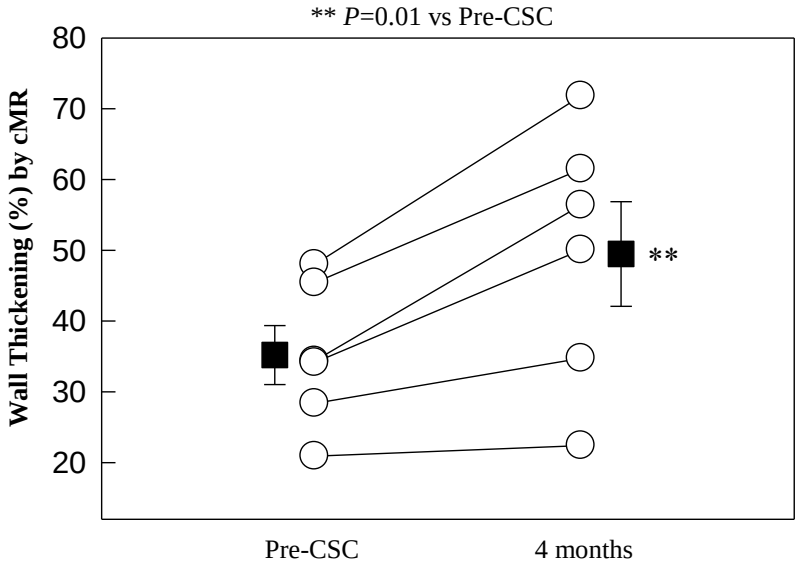
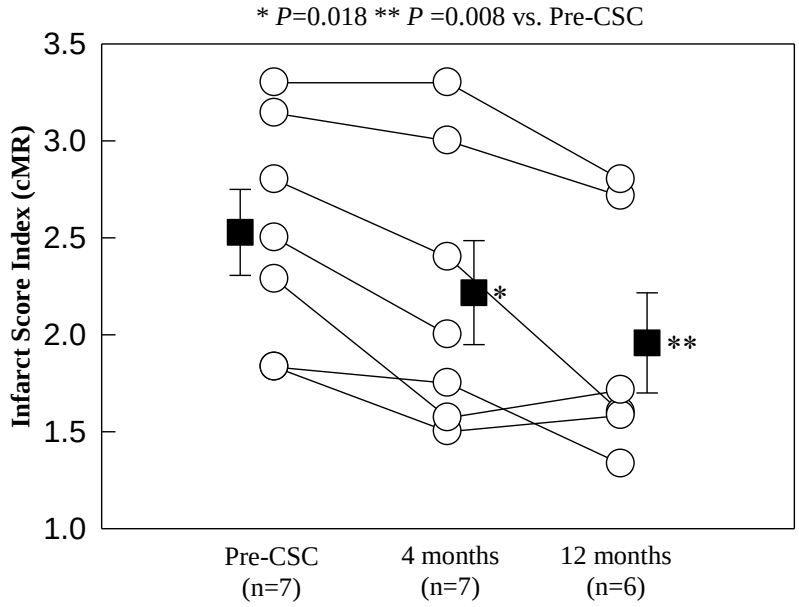


Supplemental Figure 4



Supplemental Figure 5



PROTOCOL

A. Overview of the project:

This will be an open-label study involving 20 treated patients and 20 controls. This study will be done as a collaborative project between the Brigham and Women's Hospital, Jewish Hospital/University of Louisville and Advocate Christ Medical Center. The two co-principal investigators will be Dr. Piero Anversa (who will be responsible for the studies the Brigham and Women's Hospital) and Dr. Roberto Bolli (who will be responsible for the studies at the Jewish Hospital/University of Louisville).

B. Patient enrollment criteria:

The patients that will be enrolled in the study will be patients scheduled to undergo on-pump CABG surgery and will be under the clinical care of one of the study investigators or a colleague (a cardiologist or a cardiothoracic surgeon) at Jewish Hospital, University Hospital or Advocate Christ Medical Center (depending on the clinical site at which the patient was enrolled) who has knowledge of the project. One of the study coordinators or one of the assisting research nurses will review prospectively the medical records of all patients scheduled to undergo on-pump CABG surgery to screen whether he/she satisfies the preliminary eligibility criteria for enrollment in the study. If the patient satisfies the preliminary eligibility criteria, the study coordinator will contact the patient (after communicating with the clinical care provider) and explain to him/her in detail the risks, benefits, and rationale of the study. If the patient agrees to participate in the study, the study coordinator (or the clinical care provider if the study coordinator is not available) will ask the patient to sign the informed consent form so that he/she becomes enrolled as a study subject.

Control patients will be:

a) Patients who are recruited to be controls. These are patients who are eligible for enrollment in the study but decline to receive the study treatment (intracoronary CSC injections) and consent to have the tests listed below and follow-ups through phone calls and medical records review (thereby serving as prospective controls).
b) Patients who are enrolled and are supposed to be in the treated group but cannot receive treatment because of inability to grow CSCs from their RAA.
c) Patients who are designated to the control arm of the study at the time of randomization (Stage B).

The number of control patients in the study will be 20 patients. They will meet the same eligibility criteria as the treated patients. Control patients will receive the following tests:

1) 2D & 3D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire 4±1 month post CABG

2) 2D & 3D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire 8 months post CABG

3) 2D & 3D echo, physical exam, technetium sestamibi SPECT study, low-dose echo, and Minnesota Heart Questionnaire 16 months post CABG

4) 2D & 3D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire 28 months post CABG

Preliminary inclusion/eligibility criteria (before CABG surgery):

- LVEF ≤ 40% (by any imaging modality: echocardiography/SPECT/LV angiography), and
- A history of Q-wave MI with a residual akinetic and nonviable scar (as evidenced by low-dose dobutamine stress echocardiogram and/or or thallium redistribution nuclear study for viability and/or an electrocardiogram and/or cardiac MRI and/or rest perfusion images on a sestamibi SPECT study), and

- Patients scheduled for surgical revascularization within few days (< 2 weeks) of the initial screening.
- Screening laboratory work will be performed. This will include renal function tests (serum creatinine), liver function tests (serum alkaline phosphatase, AST and ALT), and complete blood count. Patients will be deemed ineligible if the AST, ALT, alkaline phosphatase, and serum creatinine are more than 3 times the upper limit of normal, if the absolute neutrophil count (ANC) meets the WHO criteria for neutropenia (ANC < 2000) or if the total WBC number is more than two times the upper limit of normal. For hemoglobin and hematocrit, patients will be ineligible if they have Hgb< 9 g/dl and/or a hematocrit<30%. Patients with diabetes will be eligible unless their diabetic control is poor (Hgb A_{1C} is > 8.5%).

Final inclusion/eligibility criteria (just prior to CSC injection):

- Systolic LV dysfunction with EF ≤ 40% (as assessed by cardiac MRI and/or echocardiogram obtained 4 ± 1 months after CABG), and
- A history of Q-wave MI with a residual akinetic and nonviable scar (as evidenced by low-dose dobutamine stress echocardiogram and/or thallium redistribution nuclear study for viability and/or an electrocardiogram and/or cardiac MRI and/or rest perfusion images on a sestamibi SPECT study).
- Laboratory work will be performed prior to CSC injection. This will include renal function tests (serum creatinine), liver function tests (serum alkaline phosphatase, AST and ALT), and complete blood count. Patients will be deemed ineligible if the AST, ALT, alkaline phosphatase, and serum creatinine are more than 3 times the upper limit of normal, if absolute neutrophil count (ANC) meets the WHO criteria for neutropenia (ANC < 2000) or if the total WBC number is more than two times the upper limit of normal. For hemoglobin and hematocrit, patients will be ineligible if they have Hgb<9g/dl and/or a hematocrit<30%. Patients with diabetes will be eligible unless their diabetic control is poor (Hgb A_{1C} is > 8.5%).

Exclusion criteria:

- Age > 75 years old
- Cardiogenic shock
- Severe co-morbidities (e.g., renal failure, liver failure, life expectancy <12 months)
- Mini-CABG procedures
- Pregnant/nursing women or women of child-bearing potential (menstrual period in the last 12 months)
- Inability to provide informed consent
- AST, ALT, alkaline phosphatase, and serum creatinine more than 3 times the upper limit of normal
- Absolute neutrophil count (ANC) meets the WHO criteria for neutropenia (ANC < 2000) or the total WBC count is more than two times the upper limit of normal
- Hemoglobin <9 g/dl and hematocrit<30%
- Diabetics with poor control (Hgb A_{1C}> 8.5%)
- Due to the possibility of the CSCs causing arrhythmias, patients will be excluded if they have had ventricular tachycardia during the past 90 days prior to the time when CSC injection would be scheduled. Implantation of an ICD does not disqualify the patient, because the ICD will protect against any potential arrhythmias.
- Patients with a history of hepatitis B, hepatitis C, and HIV
- Patients will be excluded if they exhibit loss of mental competency after CABG

C. Study protocol:

- This study will be a phase I trial assessing the safety and feasibility of intracoronary autologous CSC (harvested from the RAA) transplantation in patients with ischemic cardiomyopathy. The study will be conducted in two stages: Stage A, in which we will obtain an initial assessment of safety and feasibility, and stage B, in which we will randomize patients.

- All patients who are undergoing on-pump CABG will be screened twice. The initial screening will be done to determine the preliminary eligibility (before-CABG screening) of patients for the study. If the patients satisfy the preliminary eligibility criteria stated above, RAA resection (which is performed as routine clinical practice during on-pump CABG) will be performed and the RAA collected, cut, and frozen in the operating room (at either Jewish Hospital, University of Louisville Hoapital or Advocate Christ Medical Center), then cultured and expanded into CSCs *in vitro*. The second screening will occur an average of 4 ± 1 month after CABG surgery, and will utilize an LVEF of ≤ 40% assessed by cardiac MRI and/or echocardiography, for final enrollment. Cardiac MRI, sestamibi SPECT, and dobutamine echo will also be performed at 4 months after CABG, prior to CSC injection.

- The preliminary eligibility criteria will utilize an EF ≤ 40% measured with any of the following cardiac imaging tests performed within 2 weeks prior to screening: echocardiography, and/or gated SPECT and/or LV angiography.

- A maximum of 100 patients who satisfy the preliminary eligibility criteria will be enrolled in the preliminary phase of the study (i.e., will have RAA tissue harvested/cultured/expanded). Of these 100 patients, a maximum of 20 will be enrolled in the final phase of the study (i.e., will undergo CSC injections and subsequent follow-up).

- A screening log will be maintained of all patients who were screened, and who satisfied the preliminary and final eligibility criteria, including detailed documentation of adverse outcomes and particularly any side effects related to the additional studies required by the research protocol.

- Enrollment of patients will be done in two stages: Stage A and stage B.

- In stage A, 9 consecutive patients will be enrolled in the treatment arm followed by 4 consecutive patients in the control arm. Stage A will enable us to perform an initial assessment of whether the process of harvesting, processing, and administering cardiac stem cells is associated with common/frequent short-term adverse effects. We will examine the possible occurrence of severe (grade 3 and/or grade 4) cardiac and arrhythmia toxicity as defined by the Common Terminology Criteria for Adverse Events (Table 5-CTCAC v 4.0; October 1, 2009) and of severe adverse events as defined in the IND application (i.e., cardiac tumors; systemic infection, myocardial infarction, sudden death [see IND application]). If severe toxicity or severe adverse events as defined above are noted, stage B will not be carried out until the FDA and IRB have reviewed the data and given permission to proceed.

- In stage B, patients will be randomized to the treated and control arms using a 2:3 ratio with a block size of 5 for the first 5 blocks. The final randomized block will be variable with a non-fixed treated-to-control ratio to ensure that the randomization arms from which patients have dropped out can be filled subsequently to adhere to the goal of 11 treated patients and 16 control patients in stage B. For instance, if three patients from both the treated and the control arms dropped out prior to the final enrollment, the final randomization will have a treated-to-control ratio of 1:1 consisting of a block of eight. (i.e. 6 drop-out patients, 2 remaining from the initial randomization). This adaptive model minimizes any bias. As a result, the total number of both treated and control patients will be 20 each. More specifically, 9 treated and 4 control patients will make up stage A and 11 treated and 16 control patients will make up stage B. To ensure that randomization can be done at the time of final enrollment, right atrial tissue will be harvested and cardiac stem cells will be grown from all patients who meet the enrollment criteria at the time of initial enrollment. If a patient is then randomized to the control arm, his/her cells will be discarded.

- In this open-label, randomized study, a maximum of 20 patients will eventually receive intracoronary CSC transplantation. These patients will have nonviable myocardium/scar from prior MI and will undergo CABG for ischemic cardiomyopathy. Control patients (a total of 20) will be (i) patients who are recruited to be controls (these are patients who are eligible for enrollment in the study but decline to receive the study treatment [intracoronary CSC injections] and consent to have the tests listed above and follow-up through phone calls and medical records review [thereby serving as prospective controls]), or (ii) patients who are enrolled and are supposed to be in the treated group but cannot receive treatment because of inability to grow CSCs from their

RAA, or (iii) patients who are designated to the control arm at the time of randomization (Stage B). Control patients will meet the same eligibility criteria as the treated patients.

- Patients who satisfy the preliminary eligibility criteria will be enrolled and revascularized within 2 weeks of enrollment.

- Patients will receive venous and arterial grafts as needed during CABG surgery.

- During on-pump CABG surgery, patients will undergo resection of part of the RAA at the cannulation site (this is done routinely during CABG surgery). Resected RAA tissue ($\leq$ 1 g) will be collected intra-operatively.

- Cardiac catheterization (coronary angiography) for intracoronary injection of CSCs will be performed 4 ± 1 month after CABG surgery.

- The methodology of intracoronary injection of CSCs will be as follows:

  1) Each patient will receive a total of 500-1,000 x 10^3 CSCs

  2) CSCs will be infused only through arteries/grafts supplying non-viable myocardial segments/scars (as detected on the 4-month viability studies performed at baseline [before CABG] and at 4 months after CABG).

  3) Injections will be performed in bypass grafts supplying coronary arteries or in native re-canalized and patent coronary arteries whether or not they were bypassed. If the graft is patent, in general the injections will be performed in the ostia of bypass grafts supplying the infarcted region. However, in the following instances, injections will be performed in the native coronary arteries, distal to the anastomosis site between the graft and the native coronary artery:

     i) If the LIMA has one or more intercostal branches, the balloon will be advanced to the native LAD and CSCs will be infused into the native LAD.

     ii.) If a graft (either LIMA or saphenous vein) supplies both the infarct-related artery and another artery that perfuses viable myocardial segments, CSCs will be selectively injected in the infarct–relate artery, thereby maximizing the delivery of CSCs to dead myocardium while avoiding potential complications in viable segments. Examples of this situation are sequential LIMA or saphenous vein grafts and grafts to an artery (e.g., the distal RCA) in which one branch (e.g., the posterior descending artery) supplies infarcted tissue whereas another branch (e.g., the postero-lateral branch) supplies viable tissue.

     iii.) If a graft is a sequential graft, the balloon catheter will be advanced to the native artery associated with the infarct and CSCs will be infused in the native artery.

     iv.) If the largest balloon size available (5 mm) is not sufficient to occlude the ostium of the graft, a smaller balloon catheter of appropriate size will be advanced into the native artery associated with the infarct and CSCs will be infused in the native artery.

  4) If inflation of the balloon is not possible for whatever reason (e.g., coronary spasm, unfavorable anatomy, unusual angle of the take-off of the LIMA from the subclavian artery, small distal vessels [<1.5 mm], etc.), the CSC solution will be infused without inflating the balloon.

  5) CSC injection:
     An over-the-wire balloon catheter (Boston Scientific Quantum Maverick non-compliant balloon or Abbott Laboratories Voyager RX balloon: balloon length 6-12 mm balloon diameter: 1.5-5 mm

depending on vessel size) will be advanced into the proximal coronary artery/graft. The balloon will be inflated for 3 min using low pressures to stop coronary flow and prevent backflow, during which, CSCs will be infused distal to the occluding balloon through the central port of the catheter. The inflation will be repeated up to 4 times (maximum) with 3 min of intervening reflow. Balloon inflations will be performed at very low pressure (within a coronary artery and not a vein graft) so as not to induce any clinically significant endothelial injury/disruption. All infusions will be performed at Jewish Hospital in Louisville, KY regardless of the site of enrollment.

- Blood tests will be done prior and subsequent to CSC delivery. Specifically, serial cardiac enzyme (CK-MB, CK, troponin) measurements and serial ECGs will be obtained every 8 hours for 24 hours after the procedure; in addition, a complete blood count, cardiac enzymes, a basic metabolic panel, liver function tests will be obtained serially (at baseline [at admission, before cardiac catheterization], at 24 hours after CSC injection; at 1, 2, and 4 weeks after CSC injection, and at 4, 8 and 12 months after CSC injection). More blood tests may be done at the discretion of the treating physician if deemed clinically necessary.

- Long-term follow up will be done up to 2 years after CSC injection.

- CSC-treated patients: Patients treated with CSCs will undergo the following tests at various time points after CSC injection:

At 4 months ± 1 month after CABG (within the 3 weeks prior to CSC administration):

a) To assess regional/global LV and RV systolic function, LVEF, fractional area change, and LV dimensions, 2-D and 3-D transthoracic echocardiograms will be performed. The following analyses will also be performed: pulsed wave spectral tissue Doppler of annulus and myocardium; color tissue Doppler derived strain and strain rate imaging of LV segments; mitral valve E/A, IVRT, and color M-mode LV inflow propagation rate.
b) To assess scar size, myocardial perfusion, and ischemia, technetium sestamibi perfusion SPECT imaging will be performed in conjunction with exercise tolerance testing (ETT [[standard or modified Bruce protocol]); if the patient cannot tolerate an ETT, pharmacologic (adenosine or dobutamine) stress will be used in conjunction with sestamibi SPECT imaging. In those patients who undergo ETT as a part of the nuclear study, functional capacity will be assessed from the nuclear study.
c) To localize and quantify viable/nonviable myocardial segments and assess contractile reserve, a low-dose dobutamine stress echocardiogram will be performed.
d) To assess cardiac function and perfusion and scar size, cardiac MRI will be performed if there are no contraindications.
e) ECG will be performed
f) Blood will be drawn to measure serum electrolytes (basic metabolic panel), liver function (liver function tests), CBC, brain natriuretic peptide (BNP), and cardiac enzymes.
g) A physical exam will be performed
h) Minnesota Heart Questionnaire will be administered and NYHA class assessed

At 1 week ± 5 d after CSC administration:

a) 2-D and 3-D transthoracic echocardiograms
b) 24-h Holter monitor
c) ECG
d) Serum electrolytes (basic metabolic panel [BMP]), liver function tests, CBC, and cardiac enzymes
e) Physical exam

At 2 weeks ± 5 d after CSC administration:

a) ECG
b) Serum electrolytes (basic metabolic panel), liver function tests, CBC, and cardiac enzymes
c) Physical exam

At 4 weeks ± 5 d after CSC administration:

a) 2-D and 3-D transthoracic echocardiograms
b) 24-h Holter monitor

c) ECG
d) Serum electrolytes (basic metabolic panel [BMP]), liver function (liver function tests), CBC, and cardiac enzymes
e) Physical exam

At 4 months after CSC administration:
a) 2-D and 3-D transthoracic echocardiograms
b) Technetium sestamibi perfusion SPECT imaging with ETT or pharmacologic stress (adenosine or dobutamine)
c) Low-dose dobutamine stress echocardiogram
d) Cardiac MRI
e) ECG
f) Serum electrolytes (basic metabolic panel [BMP]), liver function tests, CBC, and BNP
g) Physical exam
h) Minnesota Heart Questionnaire and NYHA class assessment

At 8 months after CSC administration:
a) ECG.
b) Serum electrolytes (basic metabolic panel [BMP]), liver function tests, and CBC.
c) Physical Exam

At 12 months after CSC administration:
a) 2-D and 3-D transthoracic echocardiograms
b) Technetium sestamibi perfusion SPECT imaging with ETT or pharmacologic stress (adenosine or dobutamine)
c) Low-dose dobutamine stress echocardiogram
d) Cardiac MRI
e) ECG
f) Serum electrolytes (basic metabolic panel [BMP]), liver function tests, CBC, and BNP
g) Physical Exam
h) Minnesota Heart Questionnaire and NYHA class assessment

At 2 years after CSC administration:
a) 2-D and 3-D transthoracic echocardiograms
b) Technetium sestamibi perfusion SPECT imaging with ETT or pharmacologic stress (adenosine or dobutamine)
c) Low-dose dobutamine stress echocardiogram
d) Cardiac MRI
e) ECG
f) Serum electrolytes (basic metabolic panel [BMP]), liver function tests, CBC, and BNP
g) Physical exam
h) Minnesota Heart Questionnaire and NYHA class assessment

Additional examinations and tests:
- Following intracoronary delivery of CSCs, patients will be monitored closely for 24 h in the post-PCI/angiography suite at Jewish Hospital.
- The development of peri-procedural MI will be assessed by serial cardiac enzyme measurements and serial ECGs every 8 hours for 24 hours after the procedure.
- In addition to the above tests, a CBC, cardiac enzymes, serum electrolytes (basic metabolic panel [BMP]), and liver function tests will be done at 24 h after CSC injection.
- More blood tests may be done at the discretion of the treating physician if deemed clinically necessary.

- Control Patients: Patients not treated with CSCs will undergo the following tests at various time points after CABG:

At 4 months ± 1 month post CABG:

- 2-D & 3-D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire

At 8 months post CABG:

- 2-D & 3-D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire

At 16 months post CABG:

- 2-D & 3-D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire

At 28 months post CABG:

- 2-D and 3-D echo, physical exam, technetium sestamibi SPECT study, low-dose dobutamine echo, and Minnesota Heart Questionnaire