

# Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial



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## Summary

**Background** c-kit-positive, lineage-negative cardiac stem cells (CSCs) improve post-infarction left ventricular (LV) dysfunction when administered to animals. We undertook a phase 1 trial (Stem Cell Infusion in Patients with Ischemic cardiomyopathy [SCIPIO]) of autologous CSCs for the treatment of heart failure resulting from ischaemic heart disease.

**Methods** In stage A of the SCIPIO trial, patients with post-infarction LV dysfunction (ejection fraction [EF]  $\leq 40\%$ ) before coronary artery bypass grafting were consecutively enrolled in the treatment and control groups. In stage B, patients were randomly assigned to the treatment or control group in a 2:3 ratio by use of a computer-generated block randomisation scheme. 1 million autologous CSCs were administered by intracoronary infusion at a mean of 113 days (SE 4) after surgery; controls were not given any treatment. Although the study was open label, the echocardiographic analyses were masked to group assignment. The primary endpoint was short-term safety of CSCs and the secondary endpoint was efficacy. A per-protocol analysis was used. This study is registered with ClinicalTrials.gov, number NCT00474461.

**Findings** This study is still in progress. 16 patients were assigned to the treatment group and seven to the control group; no CSC-related adverse effects were reported. In 14 CSC-treated patients who were analysed, LVEF increased from 30·3% (SE 1·9) before CSC infusion to 38·5% (2·8) at 4 months after infusion ( $p=0\cdot001$ ). By contrast, in seven control patients, during the corresponding time interval, LVEF did not change (30·1% [2·4] at 4 months after CABG vs 30·2% [2·5] at 8 months after CABG). Importantly, the salutary effects of CSCs were even more pronounced at 1 year in eight patients (eg, LVEF increased by 12·3 ejection fraction units [2·1] vs baseline,  $p=0\cdot0007$ ). In the seven treated patients in whom cardiac MRI could be done, infarct size decreased from 32·6 g (6·3) by 7·8 g (1·7; 24%) at 4 months ( $p=0\cdot004$ ) and 9·8 g (3·5; 30%) at 1 year ( $p=0\cdot04$ ).

**Interpretation** These initial results in patients are very encouraging. They suggest that intracoronary infusion of autologous CSCs is effective in improving LV systolic function and reducing infarct size in patients with heart failure after myocardial infarction, and warrant further, larger, phase 2 studies.

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## Introduction

Heart failure is a common, lethal, disabling, and expensive disorder. Its prevalence in industrialised nations has reached epidemic levels (ie, about 1 million cases in the UK<sup>1</sup> and nearly 6 million in the USA<sup>2</sup>), and continues to rise. Despite advances over the past 30 years, the prognosis for patients who are admitted to hospital with heart failure remains poor, with a 5-year mortality that is nearly 50%<sup>2</sup>—worse than that for patients with breast or colon cancer.<sup>3</sup> The most common cause of heart failure in the west is ischaemic heart disease.<sup>2</sup> Available treatments do not address the fundamental problem of the loss of cardiac tissue. As a result, interest in attempts to repair the failing heart with the use of stem cells has been increasing, since this approach has the potential to regenerate dead myocardium and thus alleviate the underlying cause of heart failure.<sup>4</sup>

The adult heart contains cardiac stem cells (CSCs) that express the surface receptor tyrosine kinase c-kit.<sup>5-7</sup> These cells are self-renewing, clonogenic, and multipotent—ie, they differentiate into all three major cardiac lineages (myocytes, vascular smooth muscle cells, and endothelial cells).<sup>5,7-11</sup> Results of many studies have shown that transplantation of CSCs in animal models of post-myocardial-infarction heart failure attenuates left ventricular (LV) remodelling and improves LV function in the settings of acute and chronic myocardial infarctions.<sup>5,7-12</sup> Despite these encouraging preclinical results, however, the effects of CSCs in patients have not been investigated. We therefore undertook a phase 1 clinical trial of CSCs in patients with heart failure after myocardial infarction to assess the safety and feasibility of intracoronary CSC infusion and to test the hypothesis that this intervention would improve the contractile

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For inclusion and exclusion criteria see <http://clinicaltrials.gov/ct2/show/NCT00474461>

See Online for webappendix

function of the heart and the general clinical status. Here we report the initial results.

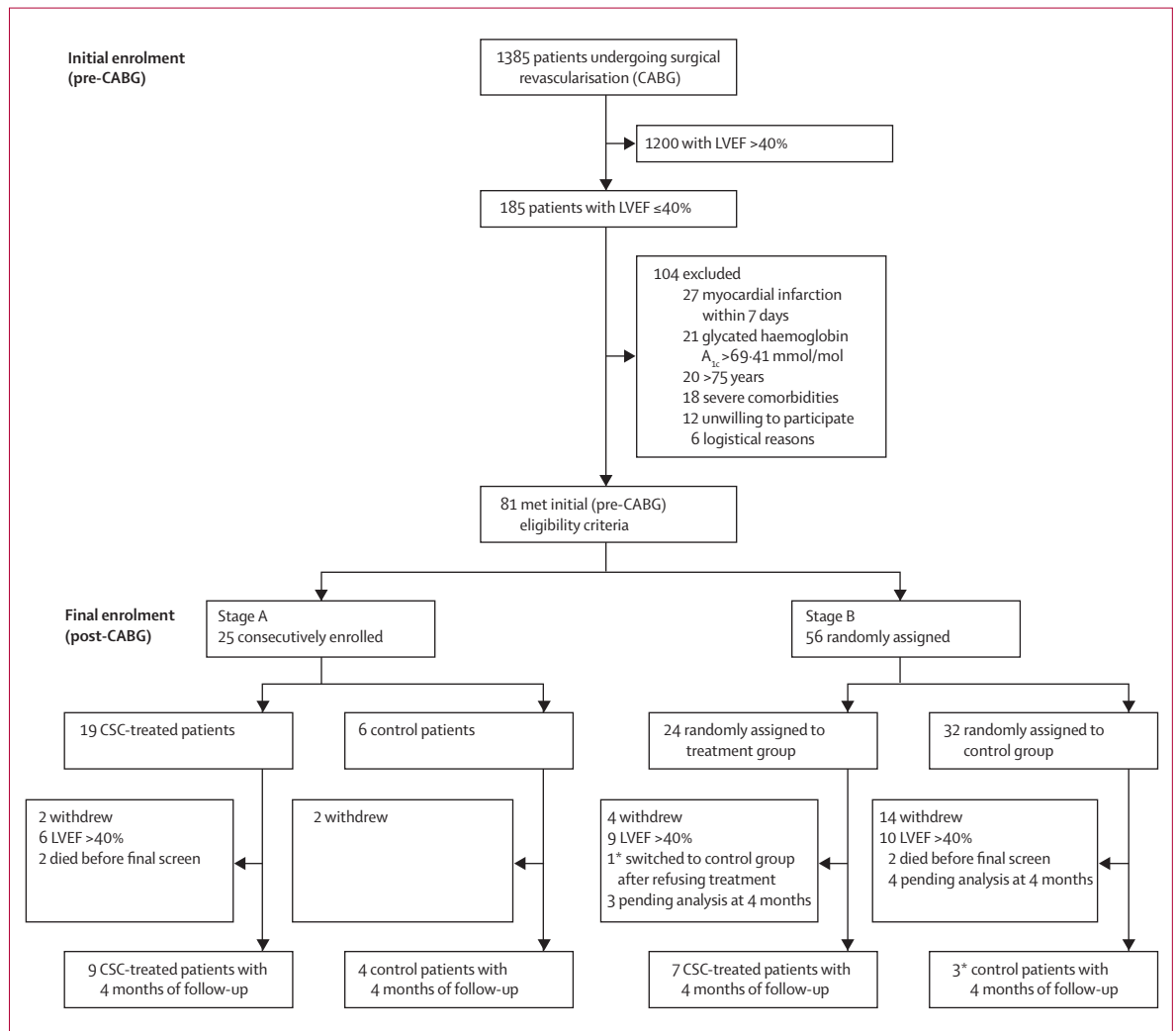
**Methods**

**Patients**

The protocol is provided in the webappendix p 14. Stem Cell Infusion in Patients with Ischemic cardiomyopathy (SCIPIO) was a phase 1, randomised, open-label, single-centre trial of the administration of autologous CSCs in patients with severe heart failure secondary to ischaemic cardiomyopathy. The target population were patients who underwent coronary artery bypass grafting (CABG), and had LV ejection fraction (EF) of less or equal to 40% and a previous myocardial infarction. Enrolment was based on eligibility screening at two timepoints. Initial screening took place within 2 weeks of CABG (figure 1). Inclusion criteria included age younger than 75 years,

LVEF less or equal to 40%, and evidence of a myocardial scar. Other inclusion and exclusion criteria are provided in the webappendix p 5. Final screening was done 3–4 months after CABG by use of the same criteria. All variables of cardiac performance obtained at final screening were regarded as baseline data.

The study protocol was reviewed and approved by the institutional review board for the University of Louisville, Louisville, KY, USA. An independent data and safety monitoring board reviewed the trial progress. Patients meeting initial eligibility criteria were approached within 2 weeks of CABG. Before enrolment, all patients agreed to participate and signed a statement of informed consent approved by the institutional review board. The study was done in accordance with the principles of the Declaration of Helsinki. Trial investigators maintained compliance with the US Food and Drug Administration



**Figure 1: Trial profile**

16 CSC-treated patients and seven control patients with 4 months of follow-up, summarising enrolment up to April 1, 2011. CABG=coronary artery bypass grafting. LVEF=left ventricular ejection fraction. CSC=cardiac stem cell. \*Patient switched from treatment group to control group.

(FDA) regulations in document 21 Code of Federal Regulations 312, subpart D, about stopping the study and the procedures to be followed in the event of severe adverse events related to the administration of CSCs (webappendix p 1).

### Randomisation and masking

SCIPIO was undertaken in two sequential stages (A and B). To assess the feasibility and short-term safety (ie, adverse effects) of CSCs, in stage A, nine consecutive patients were assigned to the treatment group followed by four consecutive patients to the control group (figure 1). In stage B, randomisation was done by two investigators (ARC and JHL) before the final eligibility screening. Numbers assigned to patients were entered into a computer software program that randomly allocated treatment assignment in a 2:3 ratio by use of an adaptive block randomisation scheme and a block size of five (figure 1). ARC and JHL assigned the patients. The purpose of adaptive block randomisation was to try to correct the imbalance between the control and the treated groups, resulting from most eligible patients wanting to be treated with CSCs. An open-label study design was used because masking would have required a cardiac catheterisation with placebo infusion in the control group. However, the investigator (MFS) doing the echocardiographic analyses was masked to group assignment.

### Study design

At the time of CABG, the right atrial appendage was harvested at the Jewish Hospital, Louisville, KY, USA, and University of Louisville and shipped to the Brigham and Women's Hospital, Boston, MA, USA, where CSCs were isolated and expanded as described below. The nearly final CSC product was transported to a Good Manufacturing Practice laboratory in Louisville for sterility testing, cell count, and assessment of viability. After confirmation of negative microbial testing, the final CSC product was prepared in 12 mL PlasmaLyte A (Baxter Healthcare, Deerfield, IL, USA) for infusion at the Jewish Hospital.

In patients assigned to the treatment group, autologous CSCs were administered by intracoronary infusion at a mean of 113 days (SE 4) after CABG. An over-the-wire balloon catheter (Quantum Maverick non-compliant balloon, Boston Scientific, Natick, MA, USA, or Voyager RX balloon, Abbott Laboratories, Abbott Park, IL, USA) was advanced into the proximal coronary artery or graft supplying the infarcted LV region. The balloon was inflated for 3 min by use of low pressure to stop coronary flow during which time CSCs were infused distally through the central port of the catheter. Four inflations with 3 min of intervening reflow were done. The number of CSCs infused depended on the number and location of the infarcts. In patients with one myocardial scar, 1 million cells were infused into anterior wall infarcts

and 500 000 cells into infarcts within the left circumflex or right coronary artery territories. In patients with several regions of infarction, 500 000 cells were infused into two different vascular territories so as not to exceed a total of 1 million cells. After infusion of CSCs, patients were monitored during an overnight stay in hospital. Patients in the control group did not undergo cardiac catheterisation.

In treated patients, two-dimensional (2D) and three-dimensional (3D) transthoracic echocardiograms, routine laboratory tests, physical examination, and New York Heart Association (NYHA)<sup>13</sup> class assessment were done before CSC infusion and at 1 month, 4 months, and 12 months thereafter. Additionally, routine laboratory tests and physical examination were done at 24 h, 1 week, 2 weeks, and 8 months after infusion of CSCs. The Minnesota Living with Heart Failure Questionnaire (MLHFQ) was completed by patients before CSC infusion and after 4 months and 12 months. In patients without contraindications, cardiac MRI (cMRI) was done before infusion of CSCs and 4 months and 12 months thereafter. A 24 h ambulatory monitor was used for detection of arrhythmias at 1 week and 4 weeks after CSC infusion.

### Isolation and expansion of CSCs

The atrial samples were cut into small pieces (<1 mm<sup>3</sup>) and suspended in 2–5 mL Ham's F12 medium (Cambrex, East Rutherford, NJ, USA) containing 1–3 mg/mL collagenase NB 6 (Crescent Chemicals, Islandia, NY, USA). After digestion, cells were plated in petri dishes containing Ham's F12 medium supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), 100 ng/mL recombinant human basic fibroblast growth factor (PeproTech, Rocky Hill, NJ, USA), 0.2 mmol/L L-glutathione (Sigma-Aldrich, St Louis, MO, USA), and 5 mU/mL human erythropoietin (Sigma-Aldrich). Subsequently, cells were expanded and subjected to immunomagnetic sorting with microbeads (human CD117 MicroBead kit, Miltenyi Biotech, Auburn, CA, USA) to obtain c-kit-positive CSCs.<sup>7,14</sup> About 2 million CSCs were obtained per patient.

### Characterisation of CSCs

To measure the fraction of c-kit-positive, lineage-negative cells in the preparation, a small sample of CSCs was fixed in 4% paraformaldehyde and incubated for 45 min at 37°C with a c-kit antibody or a cocktail of primary antibodies recognising myocytes (GATA4, Nkx2.5, Mef2c [all three Abcam, Cambridge, MA],  $\alpha$ -sarcomeric actin, connexin 43 [both Sigma-Aldrich], smooth muscle cells ( $\alpha$ -smooth muscle actin, Sigma-Aldrich), and endothelial cells (von Willebrand factor, DAKO, Carpinteria, CA, USA). Fluorescence-activated cell sorting (FACS) analysis was done with FACSaria (Becton Dickinson, Franklin Lakes, NJ, USA) or Accuri C6 (Accuri Cytometers,

For the document 21 Code of Federal Regulations 312, subpart D see <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=312>

For the Minnesota Living with Heart Failure Questionnaire see [http://www.license.umn.edu/Products/Minnesota-Living-With-Heart-Failure-Questionnaire\\_Z94019.aspx](http://www.license.umn.edu/Products/Minnesota-Living-With-Heart-Failure-Questionnaire_Z94019.aspx)

Ann Arbor, MI, USA) instruments.<sup>5,7,14</sup> Quantitative measurements of telomere length were made by use of quantitative fluorescence in situ hybridisation and confocal microscopy or flow fluorescent in-situ hybridisation (flow-FISH).<sup>7,14,15</sup> The catalytic activity of telomerase was assessed by use of quantitative PCR.<sup>14,15</sup> To measure population doubling time, CSCs were plated at low density (about 700/cm<sup>2</sup>) and the number of cells was counted daily. Population doubling time was computed by use of linear regression of log<sub>2</sub> values of cell numbers. To assess the fraction of cells that were in senescence and irreversible growth arrest, cultures were stained for the senescence-associated protein p16<sup>INK4a</sup>.<sup>7,14,15</sup> More details about these methods are provided in the webappendix p 1.

## Echocardiography

Full-volume real-time 3D echocardiography images were obtained from an apical window. The entire LV was included for volumetric measurement by full-volume 3D datasets acquired by combining four electrocardiogram (ECG)-gated pyramidal subvolumes. Images were acquired over four cardiac cycles with a matrix array ultrasonographic transducer (X3-1, Philips Medical Systems, Bothell, WA, USA). Measurements of 3D volumes and EF were done offline with a semi-automated algorithm by QLAB (version 3.1, Philips Medical Systems). From an apical full-volume acquisition, frames for LV end-diastolic volume and LV end-systolic volume measurement were identified. Endocardial contour tracing was undertaken with a semiautomatic border detection algorithm and manually adjusted if needed: after identification of the apex and mitral annulus on four-chamber and two-chamber slices, a preconfigured ellipse was automatically fitted to the endocardial borders of each frame and manually adjusted as required in appropriate planes. LV end-diastolic volume and LV end-systolic volume were measured from the 3D volumes, and the EF was derived. All measurements were done by an experienced echocardiographer (MFS). Wall motion analysis was done by use of the 16-segment model from standard parasternal and apical 2D echocardiographic views as recommended by the American Society for Echocardiography.<sup>16</sup>

## cMRI

cMR images were acquired by use of a 1.5T Espree system (Siemens Medical Solutions, Erlangen, Germany). Cardiac-gated, TrueFISP Cine (Siemens Medical Solutions) acquisitions (25 temporal frames) were done during breath holding, with phased array reception coils. Typical parameters were repetition time 5 ms, echo time 1.5 ms, flip angle 80° with a spatial resolution of 1.4 mm × 3.1 mm in plane. Default slice thickness was 8 mm, with ten to 12 short-axis image sections for complete coverage of the left ventricle. Late gadolinium enhancement for infarct assessment was also done with Multihance (Bracco, Milan, Italy) at 0.2 mmol/L per kg. Typical acquisition entailed a phase-sensitive inversion recovery technique with a spatial resolution 2.1 × 2.1 × 8.0 mm<sup>3</sup>. Post-processing was done with QMass software (version 7.2). Assessment of infarct size was done both semiquantitatively (with a standard transmural categorisation score<sup>17</sup> of 1–4, with 1 representing no infarct, 2 less than 25% transmural involvement, 3 25–50%, 4 more than 50%) and quantitatively with manual delineation in a slice-by-slice analysis with infarct tissue expressed in g.<sup>18</sup>

## Statistical analysis

The primary endpoint was the safety and feasibility of autologous CSCs for the treatment of heart failure resulting from ischaemic heart disease. The secondary endpoint was

	Treatment group (n=16)	Control group (n=7)
Age (years)	56.0 (2.2)	57.3 (3.4)
Ethnic origin		
White	15 (94%)	6 (86%)
African-American	1 (6%)	1 (14%)
Male sex	14 (88%)	7 (100%)
Body-mass index (kg/m <sup>2</sup> )	29.2 (1.1)	26.6 (1.9)
Diabetes mellitus	3 (19%)	3 (43%)
Hypertension	13 (81%)	6 (86%)
Hyperlipidaemia	10 (63%)	6 (86%)
Tobacco use		
At time of coronary artery bypass grafting	7 (44%)	7 (100%)
At time of enrolment	4 (25%)	3 (43%)
Positive family history of ischaemic heart disease	7 (44%)	6 (86%)
Baseline ejection fraction (ejection fraction units)	31.4 (1.8)	30.0 (2.3)
Arteries with stenosis greater than 50%	2.9 (0.2)	2.6 (0.2)
Infarct artery		
Right coronary artery	10 (63%)	4 (57%)
Left anterior descending artery	12 (75%)	6 (86%)
Left circumflex artery	6 (38%)	0
Old infarcts	1.9 (0.1)	1.6 (0.2)
Anterior infarction	12 (75%)	6 (86%)
Non-anterior infarction	4 (25%)	1 (14%)
Vessels infused	1.8 (0.1)	NA
Cells injected		
1 million	15 (94%)	NA
500 000	1 (6%)	NA
Drugs		
Aspirin	16 (100%)	6 (86%)
β blocker	13 (81%)	6 (86%)
Angiotensin-converting-enzyme inhibitor or angiotensin-receptor blocker	11 (69%)	4 (57%)
Statin	13 (81%)	6 (86%)
Clopidogrel	6 (38%)	2 (29%)
Baseline NYHA score	2.2 (0.2)	2.0 (0)
Baseline MLHFQ score	46.4 (5.2)	38.1 (10.5)

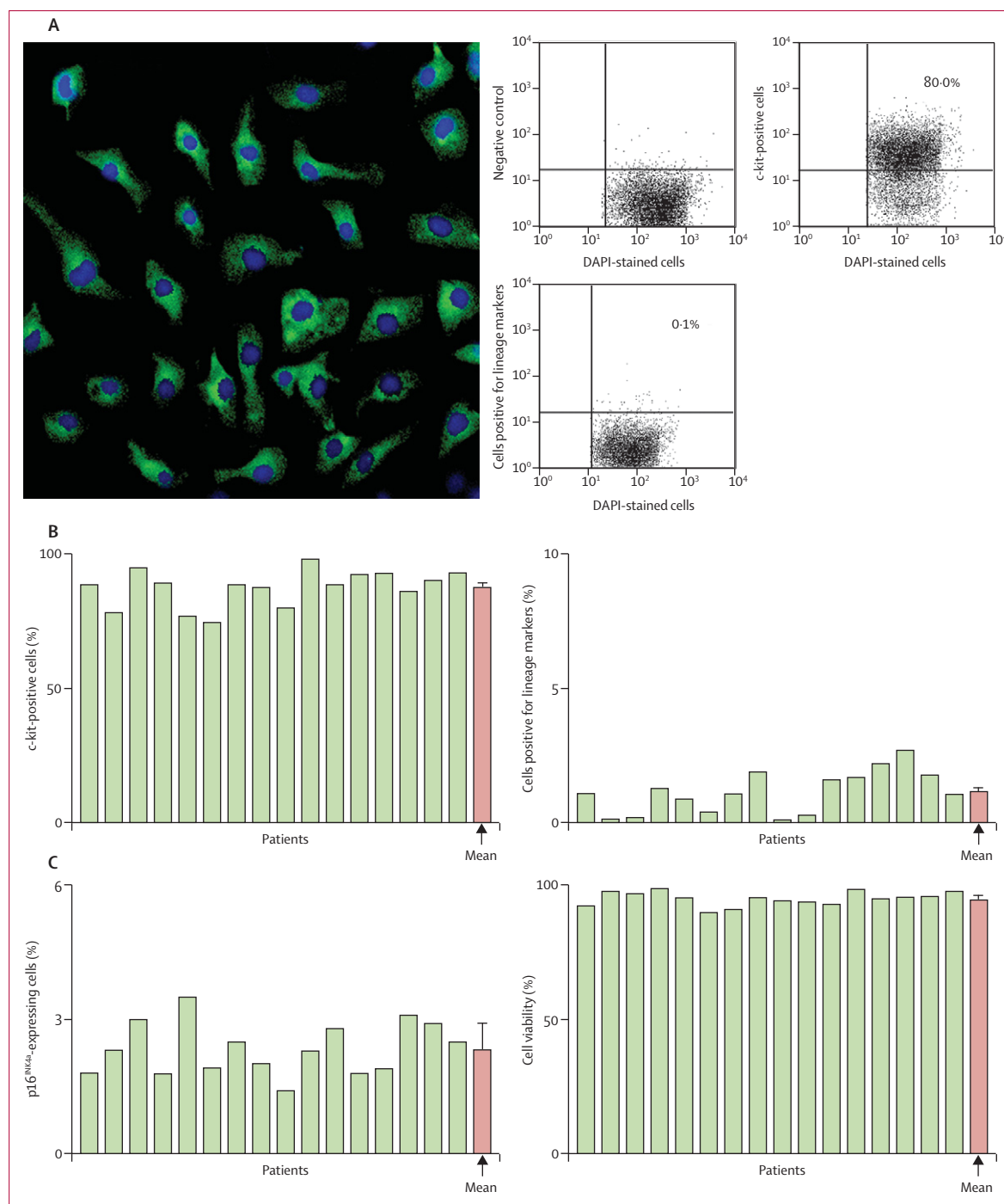
Data are number (%) or mean (SE). NA=not applicable. NYHA=New York Heart Association. MLHFQ=Minnesota Living with Heart Failure Questionnaire.

**Table 1: Characteristics of cardiac-stem-cell-treated and control patients**

the efficacy of CSCs, assessed as LV function, infarct size, and functional status. The sample size (20 treated patients) was decided in consultation with the FDA. A per-protocol analysis was used. Data are reported as mean (SE).

Comparisons between two groups were done with paired or unpaired Student's *t* tests, as appropriate.

This study is registered with ClinicalTrials.gov, number NCT00474461.



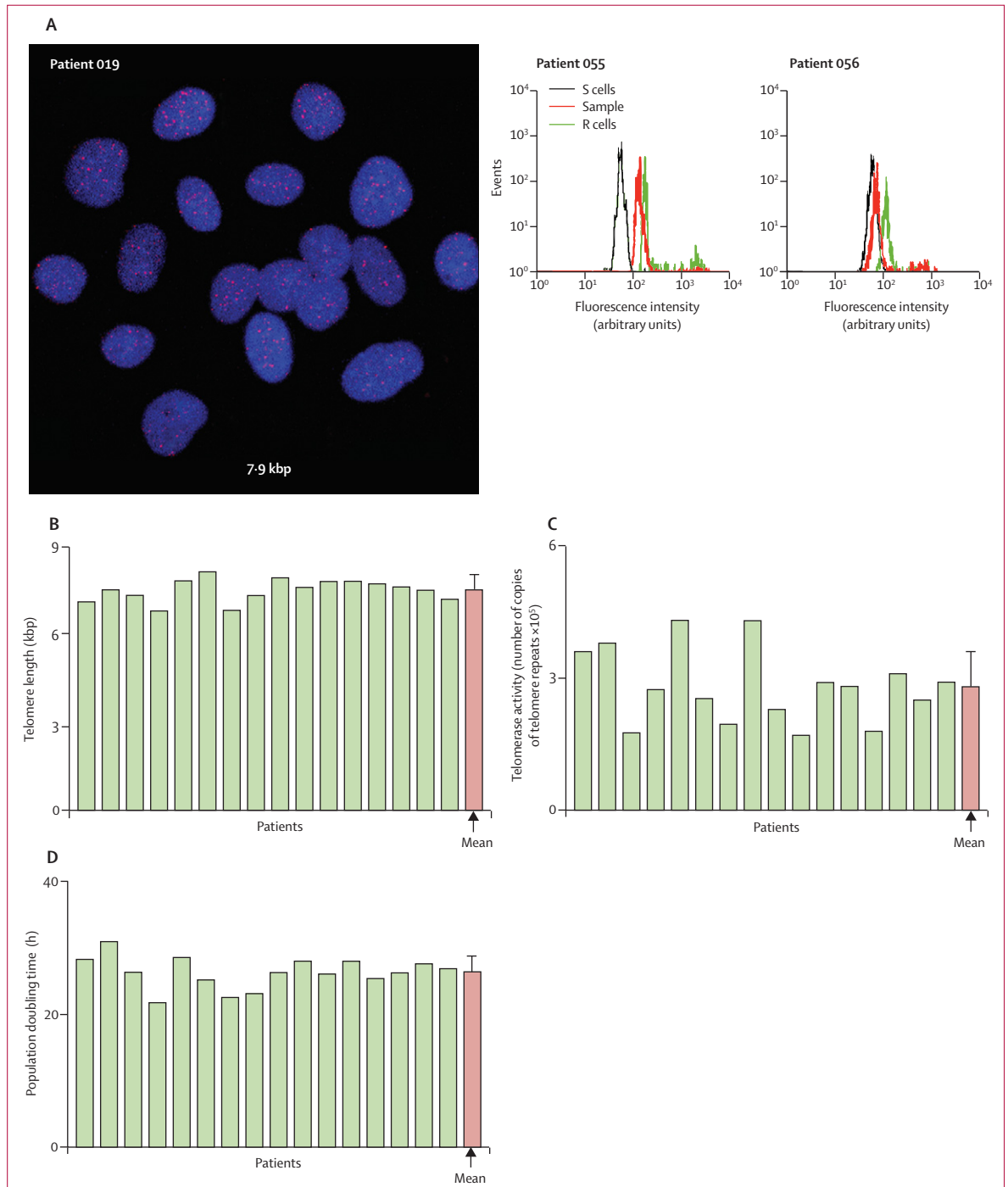
**Figure 2: Phenotype of CSCs before intracoronary administration**

(A) Confocal image showing the localisation of c-kit (green) in CSCs and the fluorescence-activated cell sorting analysis of c-kit expression and lineage markers of CSCs for patient 019. Percentages are proportions of the cell populations expressing c-kit and lineage markers. Nuclei are stained blue with DAPI. (B) Cells expressing c-kit and lineage markers of cardiac commitment. (C) Cells expressing the senescence-associated protein p16<sup>INK4a</sup> and viable cells in the final preparations. Green histograms indicate values for individual CSC-treated patients, pink histograms indicate mean (SE). CSCs=cardiac stem cells. DAPI=4'-6-diamidino-2-phenylindole.

**Role of the funding source**

The sponsors of the study had no role in study design, data collection, analysis, and interpretation, or writing

the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit the report for publication.



**Figure 3: Growth properties of CSCs before intracoronary administration**

(A) Telomeres in CSC nuclei (red dots) are identified by use of quantitative fluorescence in-situ hybridisation (patient 019) and flow FISH (patients 055 and 056). R cells with long telomeres (48 kbp) and S cells with short telomeres (7 kbp) were used to calculate absolute values; telomere length was 7.5 kbp for patient 055 and 7.2 kbp for patient 056. Graphs represent the intensity of peptide nucleic acid probe binding during flow FISH in gated CSCs (red) and control cells (green). (B) Telomere length. (C) Telomerase activity in CSC lysates from each patient was assessed by use of quantitative PCR. (D) Population doubling time of CSCs. Green bars indicate values for individual CSC-treated patients, pink histograms indicate mean (SE). CSC=cardiac stem cells. FISH=fluorescent in-situ hybridisation.



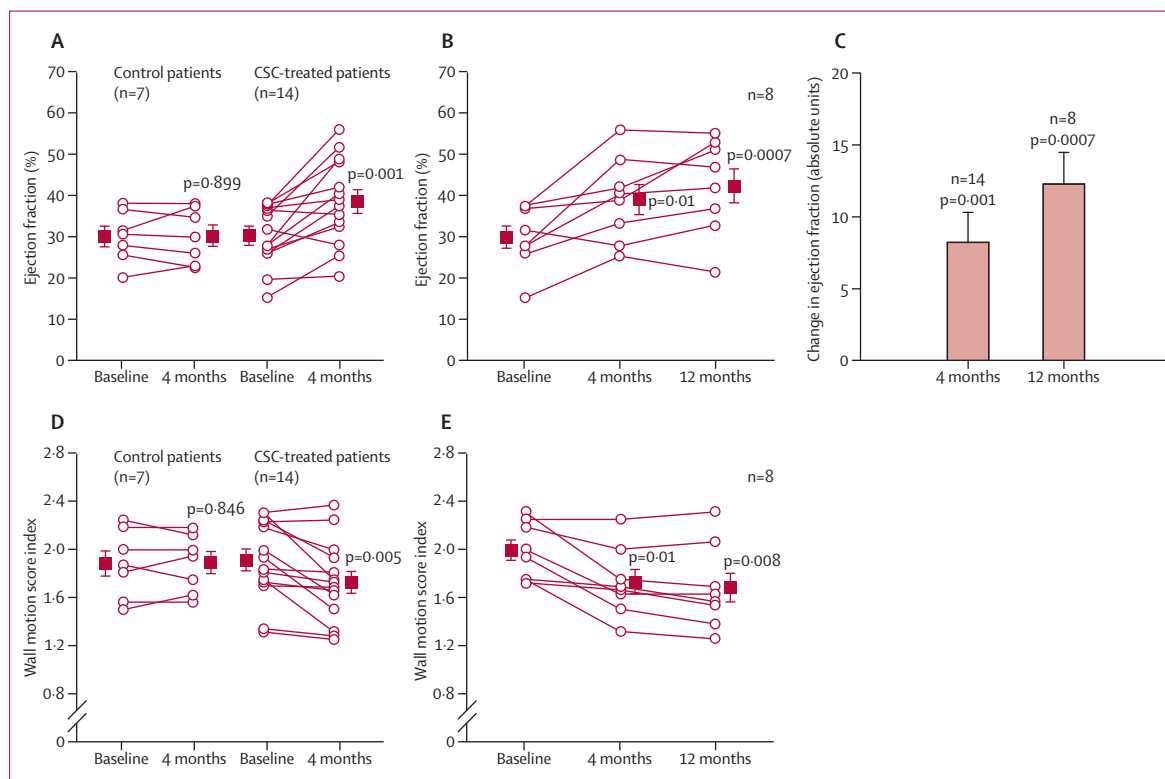
## Results

Investigational new drug approval from the FDA was obtained on Aug 8, 2008. The study was initiated in February, 2009. The first patient was enrolled on March 13, 2009, and was administered autologous CSCs on July 17, 2009. As of April 1, 2011, CSCs have been successfully isolated and expanded in 80 of 81 patients (the only failure was in a patient with cardiac amyloidosis). Figure 1 summarises the numbers of patients screened, enrolled, and excluded. The study is still in progress; here we report the interim results obtained in 16 CSC-treated and seven control patients.

Table 1 summarises the characteristics of the patients in the CSC-treated and control groups. There were no significant differences between groups at the time of CABG except for tobacco use, which was more prevalent in the control group. Analysis at the time of final enrolment (about 4 months after CABG) showed no significant differences between groups, including the use of tobacco. By design, all patients had at least one previous myocardial infarction; the mean age of the infarct was 3.7 years (SE 0.9). Five of seven control and 15 of 16 CSC-treated patients had evidence of a transmural myocardial infarction (details provided in webappendix p 2). 15 patients

were administered 1 million CSCs and one was administered 500 000 CSCs; in this patient, LVEF increased by 16.7 units at 4 months after administration of CSCs.

c-kit-positive CSCs were characterised by use of immunolabelling, confocal microscopy, and FACS analysis (figure 2A; webappendix pp 9,11). The fraction of c-kit-positive cells varied from 75.0% to 98.0% (mean 88.0% [SE 1.7]; figure 2B). CSCs committed to the myocyte, smooth muscle cell, and endothelial cell lineages constituted 0.1–2.7% of the population (1.1% [0.2]; figure 2B). Mean telomere length was 7.5 kbp (range 6.8–8.1; figure 3B; webappendix p 10), and was much higher than the lengths associated with senescence of human cells—ie, 1.5–2.0 kbp.<sup>19</sup> Additionally, telomerase activity was high in all CSC samples (figure 3C). The significant growth reserve of CSCs was confirmed by use of the population doubling time, which was never greater than 31 h (figure 3D). The well preserved telomere–telomerase axis in CSCs was consistent with the quite small percentage of CSCs that were positive for the senescence-associated protein p16<sup>INK4a</sup> (figure 2C; webappendix p 12), which permanently prevents the re-entry of stem cells into the cell cycle.<sup>20</sup> Thus, CSCs expanded in culture retained a robust capacity for further



**Figure 4: Echocardiographic analysis of CSC-treated patients and controls** (A) Left ventricular ejection fraction (measured by use of three-dimensional echocardiography) at 4 months after baseline in control and CSC-treated patients. (B) Ejection fraction at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. (C) Change in ejection fraction from baseline at 4 months and 12 months in CSC-treated patients. (D) Wall motion score index at 4 months after baseline in control and CSC-treated patients. (E) Wall motion score index at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. Boxes represent the mean values and error bars represent SE. p values are reported for difference between baseline and 4 months and between baseline and 12 months. CSC=cardiac stem cell.

cell division. There was no relation between age of the patients and either telomere length or telomerase activity (data not shown).

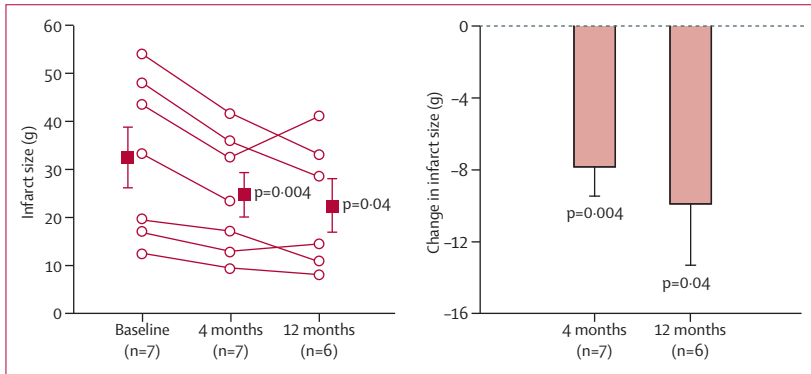
Two CSC-treated patients could not be included in the echocardiographic analysis because of poor image quality (n=1) and uncorrected aortic stenosis (n=1). In the remaining 14 treated patients, LVEF, assessed by use of

3D echocardiography, increased progressively from a mean of 30.3% (SE 1.9) before CSC infusion to 35.9% (2.7) 1 month after infusion of CSCs (p=0.014) and 38.5% (2.8) 4 months after infusion (p=0.001; figure 4A). In the eight patients who completed the 1 year of follow-up, LVEF increased further from 39.2% (3.6) at 4 months to 42.5% (4.1) at 1 year (figure 4B); although the increase from 4 months to 12 months was not significant (p=0.159), it suggests that CSCs continue to improve LV function beyond the first 4 months. The absolute increase in LVEF from baseline was 8.2 EF units (2.0) at 4 months in 14 patients and 12.3 EF units (2.1) at 12 months in eight patients (figure 4C). An improvement in LVEF was noted in 12 of 14 patients at 4 months (figure 4A) and in all eight patients who completed follow-up at 1 year (figure 4B). By contrast, in the seven control patients with 4 months of follow-up, none of these values changed much over the same time interval—eg, mean LVEF was 30.1% (2.4) at baseline (4 months after CABG) and 30.2% (2.5) at 4 months after baseline (figure 4A).

The increase in LVEF in the 14 CSC-treated patients was associated with an improvement in the regional wall motion score index, both in the infused LV regions (from a mean of 1.97 [SE 0.13] at baseline to 1.78 [0.12] at 4 months; p=0.007) and in all LV segments combined (from 1.91 [0.09] to 1.73 [0.09]; figure 4D). By contrast, in the control group there was no significant change in the regional wall motion score index at 4 months after baseline, either in infarcted LV segments (1.99 [0.09] vs 1.91 [0.09] at baseline, p=0.144) or in all LV segments combined (1.89 [0.09] vs 1.88 [0.11] at baseline; figure 4D).

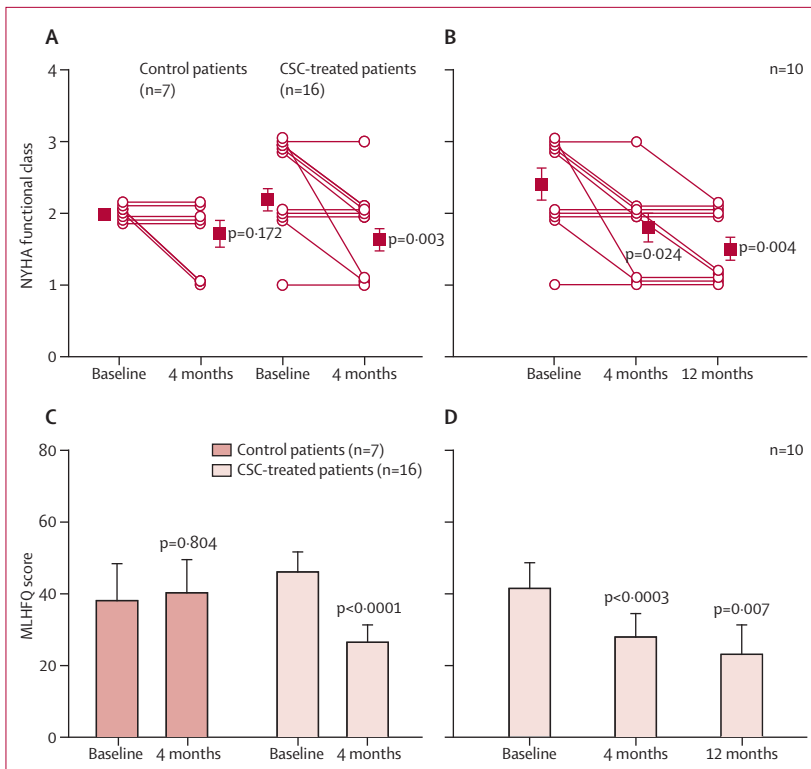
cMRI with gadolinium was undertaken in seven CSC-treated patients. Reasons for exclusion were placement of implantable cardioverter defibrillator, estimated glomerular filtration rate of less than 40 mL/min per 1.73 m<sup>2</sup>, and non-CABG postoperative status with recently placed metal hardware. The mean infarct weight, assessed with cMRI, was 32.6 g [SE 6.3] before infusion of CSCs, and decreased by 7.8 g (1.7; 24%) at 4 months after treatment and 9.8 g (3.5; 30%) at 12 months (figure 5). A reduction in infarct size was also noted with the semiquantitative infarct score index (webappendix p 13). Measurements of LV wall thickening with cMRI showed a significant (p=0.01) improvement at 4 months (webappendix p 13), confirming the echocardiographic data.

In the 16 CSC-treated patients, the NYHA functional class decreased from a mean of 2.19 (SE 0.16) before CSC infusion to 1.63 (0.16) 4 months after infusion (figure 6A). Quality of life, as assessed by use of the mean MLHFQ score, improved substantially from 46.44 (5.22) to 26.69 (4.92; figure 6C). In the seven control patients, neither the NYHA class nor the MLHFQ score changed much over the corresponding 4 months (NYHA 2.0 (0) vs 1.7 [0.2], figure 6A; and MLHFQ 38.14 [10.53] vs 40.43 [9.20], figure 6C). In ten CSC-treated patients, the improvements in NYHA (2.4 [0.2] at baseline vs 1.5 [0.2] at 12 months; figure 6B) and MLHFQ scores (41.70



**Figure 5: Infarct size and change in infarct size at 4 months and 12 months after baseline in patients administered cardiac stem cells**

p values are reported for difference between baseline and 4 months and between baseline and 12 months. Boxes and bars represent the mean values and error bars represent the SE.



**Figure 6: Functional status and quality of life in control and CSC-treated patients**

(A) NYHA functional class at 4 months after baseline in control and CSC-treated patients. (B) NYHA functional class at 4 months and 12 months after baseline in the ten CSC-treated patients who had 1 year of follow-up. (C) MLHFQ score at 4 months after baseline in control and CSC-treated patients. (D) MLHFQ score at 4 months and 12 months after baseline in the ten CSC-treated patients who had 1 year of follow-up. p values are reported for difference between baseline and 4 months and between baseline and 12 months. Bars represent the mean values and the error bars represent SE. NYHA=New York Heart Association. MLHFQ=Minnesota Living with Heart Failure Questionnaire.



	Treatment group (n=16)	Control group (n=7)
Death	0	0
Myocardial infarction (peri-procedural or post-procedural)	0	0
New tumour	0	0
Ventricular arrhythmia	0	0
Systemic infection (within 1 year)	0	0
Stroke	0	0
Allergic reaction	0	NA
Procedure-related event*	1 (6%)	NA
Revascularisation	0	1 (14%)
Hospital admission for heart failure†	1 (6%)	0
Hospital admission for angina	1 (6%)	2 (29%)

NA=not applicable. \*Tortuous left internal mammary artery engaged for cardiac-stem-cell infusion had intimal dissection after balloon deflation; drug-eluting stent was placed without complication. †Secondary to worsening valvular disease.

**Table 2: Adverse events in cardiac-stem-cell-treated and control patients**

[7.54] vs 23.50 [8.04]; figure 6D) were even more pronounced at 1 year.

No adverse effects attributable to CSCs were noted (table 2). Specifically, none of the CSC-treated patients had non-fatal myocardial infarction (immediately after CSC infusion or during follow-up), death, tumour formation, ventricular arrhythmias, systemic infection, stroke, allergic reactions, or coronary revascularisation. With 24 h ambulatory ECG monitoring on two separate occasions (1 week and 4 weeks after CSC treatment), no tachyarrhythmias were noted. One treated patient was admitted to hospital for heart failure, one treated patient and two control patients were admitted for angina, and one control patient underwent percutaneous coronary revascularisation. In one treated patient, a dissection of the left internal mammary artery graft occurred during balloon inflation; the dissection was repaired with a stent and no complications arose over the following 2 years. Information from the SCIPIO trial about the safety and feasibility of administration of CSCs to patients is further described in webappendix p 2.

## Discussion

Our results suggest that CSCs can be reproducibly isolated and expanded from about 1 g myocardial tissue that is harvested during cardiac surgery. Infusion of 1 million autologous CSCs is not associated with apparent adverse effects for up to 1 year; and infusion of autologous CSCs results in a substantial improvement in LV systolic function 4 months after infusion and an even more pronounced improvement 1 year after infusion and is associated with increased functional capacity, improved quality of life, and reduced LV scar size.

CSCs are particularly attractive for cardiovascular applications because they normally reside in the adult

heart and can be reproducibly isolated and expanded, even from endomyocardial biopsies.<sup>14</sup> These cells are thought to replenish the pool of cardiac myocytes and cardiac vascular cells that die during an organism's lifetime.<sup>5-8</sup> The initial results of SCIPIO are consistent with the salutary effects of CSCs reported in preclinical studies<sup>5,7-11</sup> and compare favourably with the results of previous clinical studies of a variety of non-cardiac stem or progenitor cells in patients with ischaemic cardiomyopathy (webappendix p 6).<sup>4</sup> In particular, the increase in LVEF in this study compares very favourably with the 3-7% improvement reported in previous studies of intracoronary infusion of bone marrow mononuclear cells in similar patient populations (panel).<sup>21-24</sup> The optimum dose of CSCs remains to be established. The dose of CSCs used in SCIPIO (1 million) was selected conservatively on the basis of preclinical studies in pigs<sup>8</sup> and the growth characteristics of the cells. Our CSC population had a mean telomere length of 7.5 kbp (figure 3B). Because 130 bp of telomeric DNA are lost after each division<sup>7</sup> and senescence occurs when telomeres reach 1.5-2.0 kbp, a single engrafted CSC could, in principle, divide 42 times before irreversible growth arrest, generating  $4 \times 10^{12}$  cells. We have shown that CSCs can be isolated and expanded from small (about 5 mg) endomyocardial biopsies.<sup>14</sup> In future studies, assessment of the effects of larger numbers of autologous CSCs will be possible and in a much wider population of patients with heart failure by use of endomyocardial biopsies. Since CSCs can be frozen for subsequent use, even better results might be obtained with repeated infusions in the same patient.

We elected to deliver CSCs at a mean of almost 4 months after CABG to allow resolution of myocardial stunning or hibernation,<sup>28</sup> and any improvement to occur in LV function secondary to revascularisation. The high number of patients who were excluded because of an increase in LVEF during the first 4 months after CABG lends support to the appropriateness of this decision (figure 1). The general agreement is that, by 4 months after CABG, stunned or hibernating myocardium has recovered and LV function is fairly stable.<sup>29-31</sup> Indeed, no improvement was noted in the seven control patients. Therefore, the improvement in LV systolic performance noted in CSC-treated patients was unlikely to be indicative of the effects of CABG.

The reduction in infarct size noted by use of cMRI is consistent with cardiac regeneration, although whether regeneration, if it occurred, was mediated by differentiation of the injected CSCs, activation of resident CSCs, or both is not known. Answering this crucial question will necessitate the development of strategies to track the long-term fate of CSCs in patients. Other mechanisms (eg, paracrine actions resulting in inhibition of apoptosis, inhibition of fibrosis, or enhanced contractile performance) cannot be excluded. Irrespective of the mechanism, it is noteworthy that autologous CSCs improved LV performance despite the presence of mature scars.

**Panel: Research in context****Systematic review**

We searched the PubMed database for original papers using the terms “c-kit”, “cardiac stem cell”, “stem cell therapy”, “bone marrow”, “myocardial infarction”, and “chronic ischemic heart failure”. No studies of c-kit-positive cardiac stem cells (CSCs) were identified in people. All in-vivo studies of CSCs were done in animal models. 12 clinical trials were found in which bone-marrow-derived cells were used for the treatment of heart failure secondary to chronic ischaemic cardiomyopathy (a setting relevant to SCPIO; webappendix p 6). In four of these studies,<sup>21–24</sup> bone marrow cells were infused intracoronarily (in one study,<sup>24</sup> they were infused during coronary artery bypass grafting [CABG]), in three studies the cells were injected transendocardially, and in five they were injected epicardially during CABG; webappendix p 6).

**Interpretation**

Our study is the first report of the administration of CSCs in people. The results are a significant addition to the current data because they introduce a new potential treatment for heart failure. This work represents the clinical translation of the results of a large number of preclinical studies showing salutary effects of CSCs on left ventricular (LV) function and structure in mouse, rat, dog, and pig models of post-myocardial infarction heart failure.<sup>25</sup> SCPIO differs from all previous trials of cell therapy for heart disease in which non-cardiac cell products (mostly bone-marrow-derived cells<sup>6</sup>) were used. Most of these previous studies were undertaken in patients with acute myocardial infarction; in few studies did the investigators seek to treat heart failure caused by chronic ischaemic cardiomyopathy, which was the setting for SCPIO. Our results indicate an increase in global LV function (LV ejection fraction [EF]) of 8 EF units at 4 months and 12 EF units at 12 months after CSC administration. Among previous studies of bone marrow cells for chronic ischaemic cardiomyopathy (webappendix p 6), investigators of the four studies in which intracoronary infusion was used reported increases in LVEF of 3 EF units (TOPCARE-CHD),<sup>21</sup> 7 EF units (IACT),<sup>22</sup> and 6 EF units (STAR-heart)<sup>23</sup> at 3 months, 5 EF units<sup>24</sup> at 6 months, and 6 EF units (STAR-heart<sup>23</sup>) at 1 year and 5 years. Of the three studies of transendocardial injection, an increase in LVEF of 5.5 EF units was reported at 2 months, and no significant improvement was reported at 6 months and 1 year in two studies<sup>26,27</sup> (webappendix p 6). Epicardial cell delivery differs greatly from intracoronary infusion, requiring a thoracotomy. Thus, although the primary purpose of our phase 1 trial was to assess the safety and feasibility of using this distinct and unique population of cells, the treatment effects are very encouraging and compare favourably with previous trials of bone marrow cells. The present results provide a strong rationale for further studies of CSC treatment in patients with severe heart failure secondary to ischaemic cardiomyopathy, who have a poor prognosis.

Limitations of SCPIO include the small number of patients and the absence of placebo-treated patients (which resulted in the open-label design). These features, which are common to many phase 1 trials, result from the novel nature of the treatment, and from the fact that masking was not possible because it would have necessitated intracoronary infusion of vehicle; nevertheless, all echocardiograms were analysed without knowledge of treatment allocation. Any potential conditioning effect of the brief coronary occlusions in treated patients would be short lived (48–72 h),<sup>28</sup> and thus could not account for the long-term benefits. We emphasise that SCPIO was designed to investigate the safety and feasibility of intracoronary CSC infusion in patients with severe heart failure, not to assess efficacy. All efficacy data need to be verified in larger studies.

In conclusion, the initial results of SCPIO suggest that intracoronary infusion of autologous CSCs in patients with chronic ischaemic cardiomyopathy and severe heart failure is feasible, safe, and apparently highly efficacious in restoring LV systolic function up to 1 year after treatment. Since SCPIO is the first study of CSCs in human beings, the results will be important for developing this new form of cell therapy. The data from our study warrant further, larger, phase 2 studies.

**Contributors**

RB was the chief investigator for the study, and designed and managed the study with input from the group. PA, DD'A, AL, TH, FS, PG, DC, DGR, and JK designed, undertook, and interpreted all experiments involving CSCs. MFS and SGW designed, undertook, and interpreted all echocardiographic studies. GMB designed, interpreted, and did all cMRI studies. SI designed and did all interventional procedures. ARC, JHL, JBE, NKS, IF, and MSS contributed to the design of the study, collection and interpretation of data, and preparation of the report. All authors participated in data interpretation. RB drafted the first and subsequent versions of this report with input and key revisions by all authors, who reviewed and approved the final submitted report.

**Conflicts of interest**

PA is a member of Autologous. The other authors declare that they have no conflicts of interest.

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