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

Roberto Bolli, Atul R Chugh, Domenico D'Amario, John H Loughran, Marcus F Stoddard, Sohail Ikram, Garth M Beache, Stephen G Wag
Annarosa Leri, Toru Hosoda, Fumihiro Sanada, Julius B Elmore, Polina Goichberg, Donato Cappetta, Naresh K Solankhi, Ibrahim Fahsa
D Gregg Rokosh, Mark S Slaughter, Jan Kajstura, Piero Anversa

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Summary

Background c-kit-positive, lineage-negative cardiac stem cells (CSCs) improve post-infarction ve cular (LV) dysfunction when administered to animals. We undertook a phase 1 trial (Stem Cell I usio atients h Ischemic cardiOmyopathy [SCIPIO]) of autologous CSCs for the treatment of heart fai resulting is emic heart disease.

Methods In stage A of the SCIPIO trial, patients with post-infarction LV dysfunction (ejec fraction [EF] ≤40%) before coronary artery bypass grafting were consecutively enrolled in the t nt and contro ups. In stage B, patients were randomly assigned to the treatment or control group in .3 ratio by use of a computer-generated block randomisation scheme. 1 million autologous CSCs were adminis d by intrac nary infusion at a mean of 113 days (SE 4) after surgery; controls were not given any treatment lthough th tudy was open label, the echocardiographic analyses were masked to group assignment. The prima dpoint s short-term safety of CSCs and the secondary endpoint was efficacy. A per-protocol analy used. This s registered with ClinicalTrials.gov, number NCT00474461.

Findings This study is still in progress. 16 patients were assig he treatment group and seven to the control group; no CSC-related adverse effects were repor CSC-t ed patients who were analysed, LVEF increased from 30·3% (SE 1·9) before CSC infusion to 3 % (2· t 4 mo after infusion (p=0·001). By contrast, in seven control patients, during the corresponding t inter VEF did ot change (30·1% [2·4] at 4 months after CABG *vs* 30·2% [2·5] at 8 months after CABG) mpo he sa ous effects of CSCs were even more pronounced at 1 year in eight patients (eg, LVEF inc ed by 12 ction fraction units [2·1] *vs* baseline, p=0·0007). In the seven treated patients in whom cardiac M ld be done rct size decreased from 32·6 g (6·3) by 7·8 g (1·7; 24%) at 4 months (p=0·004) and 9·8 g (, 30 1 year (p=0 04).

Interpretation These initi esults in patie e very encouraging. They suggest that intracoronary infusion of autologous CSCs is eff e in i oving LV systolic function and reducing infarct size in patients with heart failure after myocardial infa , a arrant further, larger, phase 2 studies.

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See Comment page 1827

Divisions of Cardiovascular Medicine (Prof R Bolli MD, A R Chugh MD, J H Loughran MD, Prof M F Stoddard MD, Prof S Ikram MD, S G Wagner MD, J B Elmore MD, N K Solankhi MD, I Fahsah MD, D G Rokosh PhD) **and Cardiothoracic Surgery** (Prof M S Slaughter MD), **and Department of Radiology** (G M Beache MD), **University of Louisville, Louisville, KY, USA; and Departments of Anesthesia and Medicine and Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA** (D D'Amario MD, A Leri MD, T Hosoda MD, F Sanada MD, P Goichberg PhD, D Cappetta PhD, J Kajstura PhD, Prof P Anversa MD)

Correspondence to:
Prof Roberto Bolli, Division of Cardiovascular Medicine, 550 S Jackson Street, Ambulatory Care Building, 3rd Floor, Louisville, KY 40202, USA
rbolli@louisville.edu

Introduction

Heart failu a al, disabling, and expensive disorder. Its valence in industrialised nations has reached epidem vels (ie, about 1 million cases in the UK[1] and nearly 6 l ion in the USA[2]), 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%[2]—worse than that for patients with breast or colon cancer.[3] The most common cause of heart failure in the west is ischaemic heart disease.[2] 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.[4]

The adult heart contains cardiac stem cells (CSCs) that express the surface receptor tyrosine kinase c-kit.[5–7] 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).[5,7–11] 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.[5,7–12] 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

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

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

See Online for webappendix

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 b[illegible].

The study protocol was [illegible]viewed an[illegible]proved by the institutional review board [illegible]he Univers[illegible] of Louisville, Louisville, KY, USA. An in[illegible]endent [illegible]a and safety monitoring boar[illegible]eviewed th[illegible]al [illegible]gress. Patients meeting initia[illegible]gibilit[illegible]teria w[illegible]pproached within 2 weeks of C[illegible]. Be[illegible] enrolment, all patients agreed to parti[illegible]ate [illegible]igned [illegible]atement of informed conse[illegible]approved [illegible]e i[illegible]utional review board. The st[illegible] done in ac[illegible]ce with the principles of the D[illegible]clarat[illegible]of Helsinki. Trial investigators maintained compliance [illegible]h the US Food and Drug Administration

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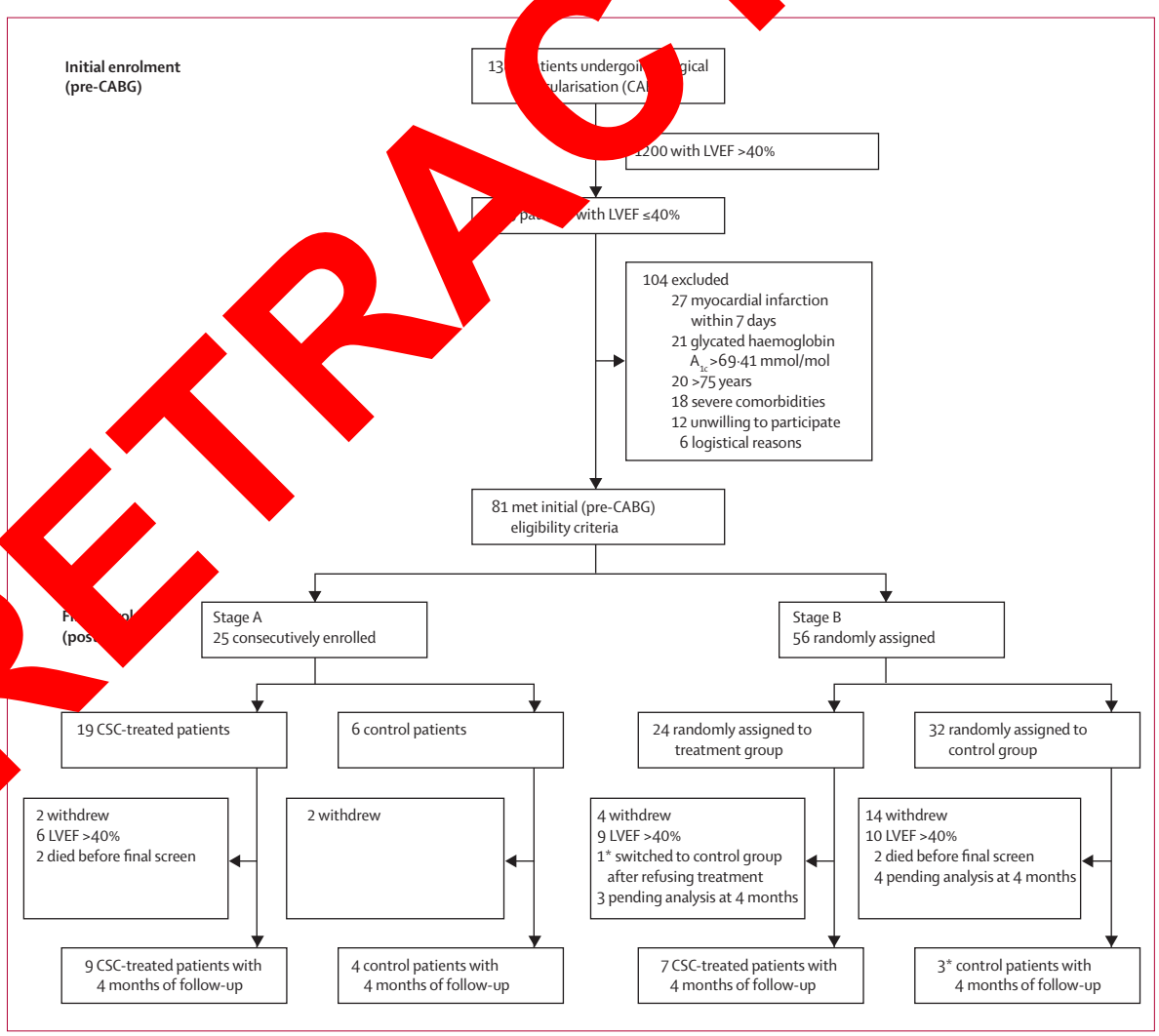

Figure 1: **Trial profile**
16 CSC-treated patients and seven control patients with 4 months of follow-up, summarising enrolment up to April 1, 2011. CABG=coronary artery bypass grafting. LVEF=left ventricular ejection fraction. CSC=cardiac stem cell. *Patient switched from treatment group to control 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 (MES) doing the echocardiographic analyses was ma group assignment.

Study design

At the time of CABG, the right atr appenda as harvested at the Jewish Hospital ville, KY, U and University of Louisville and pe the Brigham and Women's Hospital, Bos , MA, USA ere CSCs were isolated and expan as described y. The nearly final CSC pro was nsported to a Good Manufacturing Prac lab ory in Louisville for sterility testing, cell co d asses nt of viability. After confirm f neg e m obial testing, the final CSC duct prepar 2 mL PlasmaLyte A (Baxter thcare eerfield, IL, USA) for infusion at the Jewis

In patients ned to the treatment group, autologous CSCs were adm tered by intracoronary infusion at a mean of 113 days 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 h Patients in the control group did not underg rdiac catheterisation.

In treated patients, two-dimensional (2) and e-dimensional (3D) transthoracic echocar grams, rou laboratory tests, physical examina , and w Yor Heart Association (NYHA)[13] class a sm were done before CSC infusion and at mon months d 12 months thereafter. Ad nally, ro la atory tests and physical exa n were d 24 h, 1 week, 2 weeks, and mont fter infusion of CSCs. The Minnesota Living with Hea lure Questionnaire (MLHFQ) wa pleted by pa ts before CSC infusion and er 4 months and 12 months. In patients without co indication ardiac MRI (cMRI) was done before fusion of SCs and 4 months and 12 months the er. A n ambulatory monitor was detection ythmias at 1 week and 4 weeks a sion.

Isola d expansion of CSCs

The a l samples were cut into small pieces (<1 mm³) and s ended in 2–5 mL Ham's F12 medium (Cambrex, East Rutherford, NJ, USA) containing ng/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.[7,14] 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], α-sarcomeric actin, connexin 43 [both Sigma-Aldrich), smooth muscle cells (α-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

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Ann Arbor, MI, USA) instruments.[5,7,14] 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).[7,14,15] The catalytic activity of telomerase was assessed by use of quantitative PCR.[14,15] To measure population doubling time, CSCs were plated at low density (about 700/cm^2) and the number of cells was counted daily. Population doubling time was computed by use of linear regression of $\log_2$ 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^{INK4a}.[7,14,15] More details about these methods are provided in the webappendix p 1.

	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^2)	29·2 (1·1)	
Diabetes mellitus	3 (19%)	[illegible]%)
Hypertension	13 (81%)	6 [illegible]
Hyperlipidaemia	10 (63%)	6 (8[illegible]
Tobacco use		
At time of coronary artery bypass grafting	7 (44%)	7 (100%
At time of enrolment	4 (25%)	[illegible]
Positive family history of ischaemic heart disease	[illegible]4%)	6 (86%)
Baseline ejection fraction (ejection fraction units)	[illegible]·4 (1·8)	30·0 (2·3)
Arteries with stenosis greater than 50%	[illegible]0·2)	2·6 (0·2)
Infarct artery		
Right coronary artery	10 (63[illegible]	4 (57%)
Left anterior descending artery	12 (75%)	6 (86%)
Left circumflex artery	6 (38%)	0
Old infarcts	[illegible](0·1)	1·6 (0·2)
Anterior infarction	[illegible] (75%)	6 (86%)
Non-anterior inf[illegible]	4 (25%)	1 (14%)
Vessels infus[illegible]	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**

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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 s[illegible] Images were acquired over four cardi[illegible]cycles wi[illegible] matrix array ultrasonographic transd[illegible] (X3-1, [illegible]ips Medical Systems, Bothell, [illegible]VA, [illegible]A). Me[illegible]rements of 3D volumes and [illegible] were d[illegible]off[illegible] with a semi-automated al[illegible]thm [illegible]QLAB[illegible]sion 3.1, Philips Medical Syst[illegible] Fr[illegible]n apical full-volume acquisition, frames f[illegible] LV [illegible]stolic [illegible]e and LV end-systolic volu[illegible] measure[illegible] [illegible] identified. Endocardial co[illegible]acing was [illegible]taken with a semiautomatic t[illegible]der [illegible]tion algorithm and manually adjusted if needed: aft[illegible]ntification of the apex and mitral annulus [illegible]n four-chamb[illegible]nd two-chamber slices, a preconfigured ellipse was automatically fitted to the endocardial borders of e[illegible] frame and manually adjusted as required in appro[illegible]ate planes. LV end-diastolic volume and LV end-syst[illegible] volume were measured from the 3D volumes, [illegible]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.[16]

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^3. Post-processing was done with QMass software (version 7.2). Assessment of infarct size was done both semiquantitatively (with a standard transmural categorisation score[17] 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.[18]

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

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.

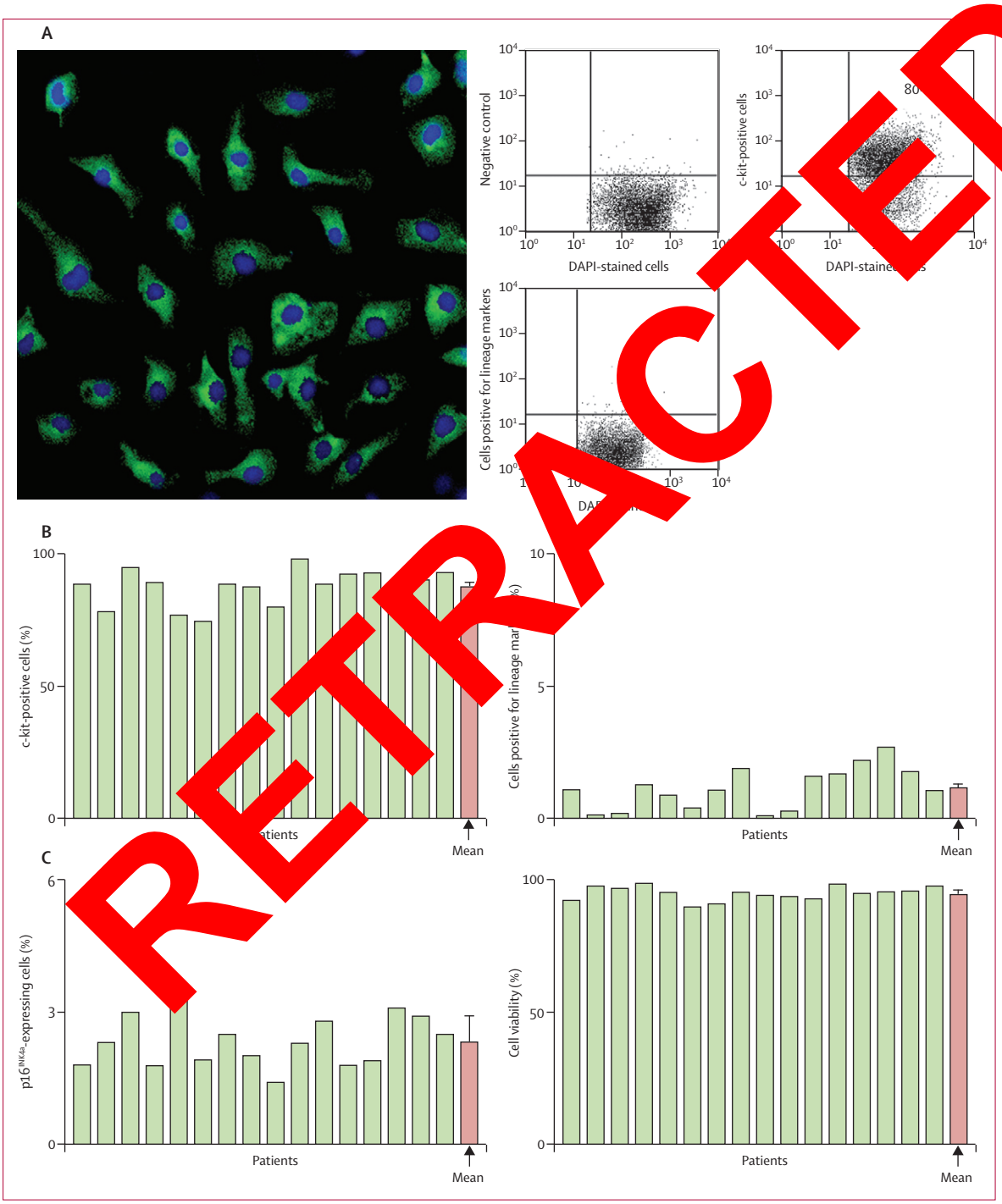

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Figure 2: **Phenotype of CSCs before intracoronary administration**
(A) Confocal image showing the localisation of c-kit (green) in CSCs and the fluorescence-activated cell sorting analysis of c-kit expression and lineage markers of CSCs for patient 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 $p16^{INK4a}$ 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.

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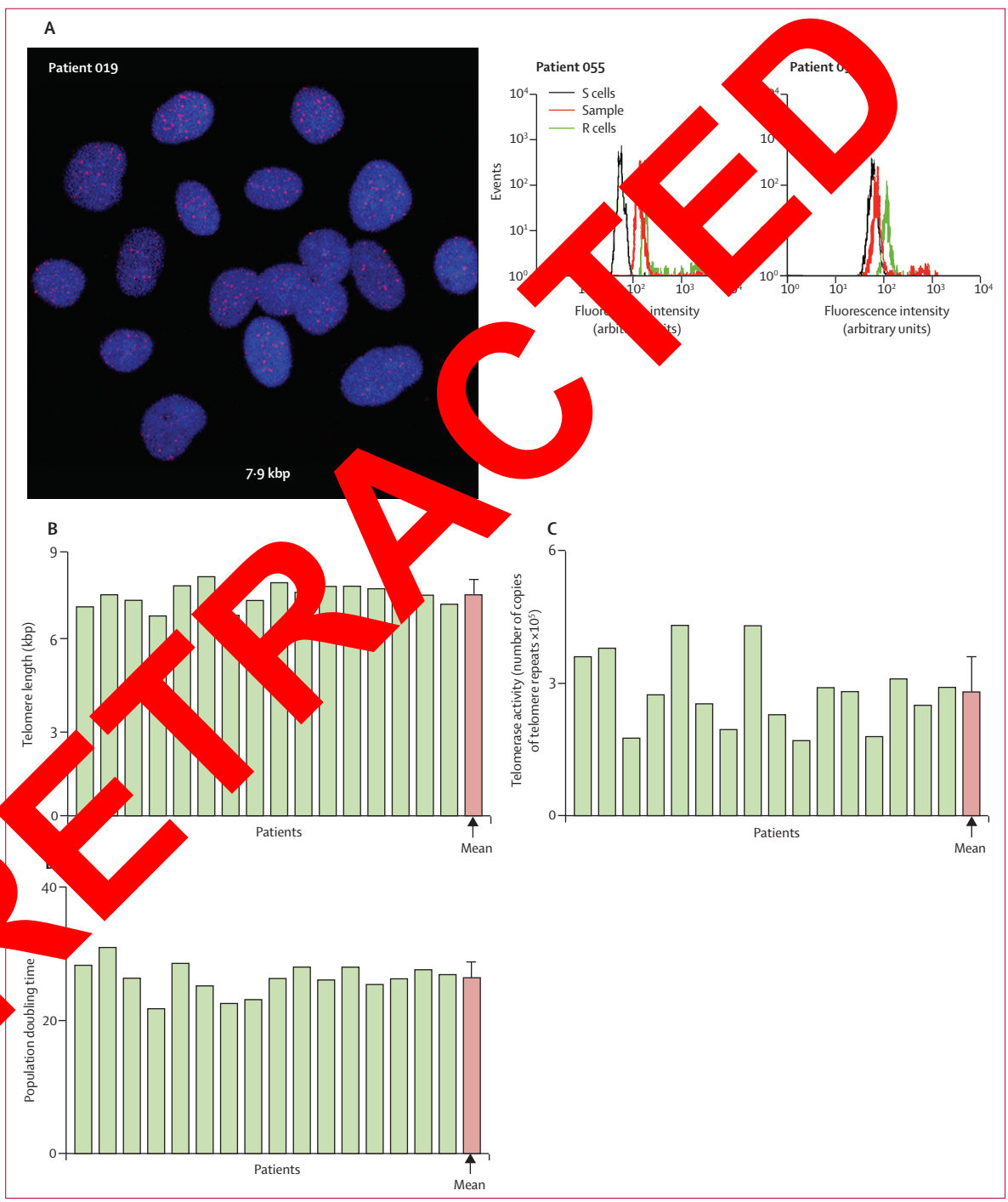

Figure 3: **Growth properties of CSCs before intracoronary administration**

(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 f… of c-kit-positive cells varied from 75·0% t… 3·0% (mean 88·0% [SE 1·7]; figure 2B). CSCs commi… to the myocyte, smooth muscle cell, and endothe… cell l… es constituted 0·1–2·7% of the popula… (1·1% [… figure 2B). Mean telomere le… was … 5 kb… (range 6·8–8·1; figure 3B; webapp… ix p…, and was much higher than the lengths … ocia… n senes… e of human cells—ie, 1… 2·0 kbp. Addi… ally, telomerase activity wa… in all C… amples (figure 3C). The signi… nt g… reserve of CSCs was confirmed by use of the populatio… ubling time, which was never great… 31 h (figure 3… he well preserved telomere–telo… erase axis in CSCs was consistent with the quite sm… percentage… CSCs that were positive for the senescen… associated … otein p16^{INK4a} (figure 2C; webappendix p… which… rmanently prevents the re-e… f stem ce… the cell cycle.[20] Thus, CSCs e… ad… ulture retained a robust capacity for further

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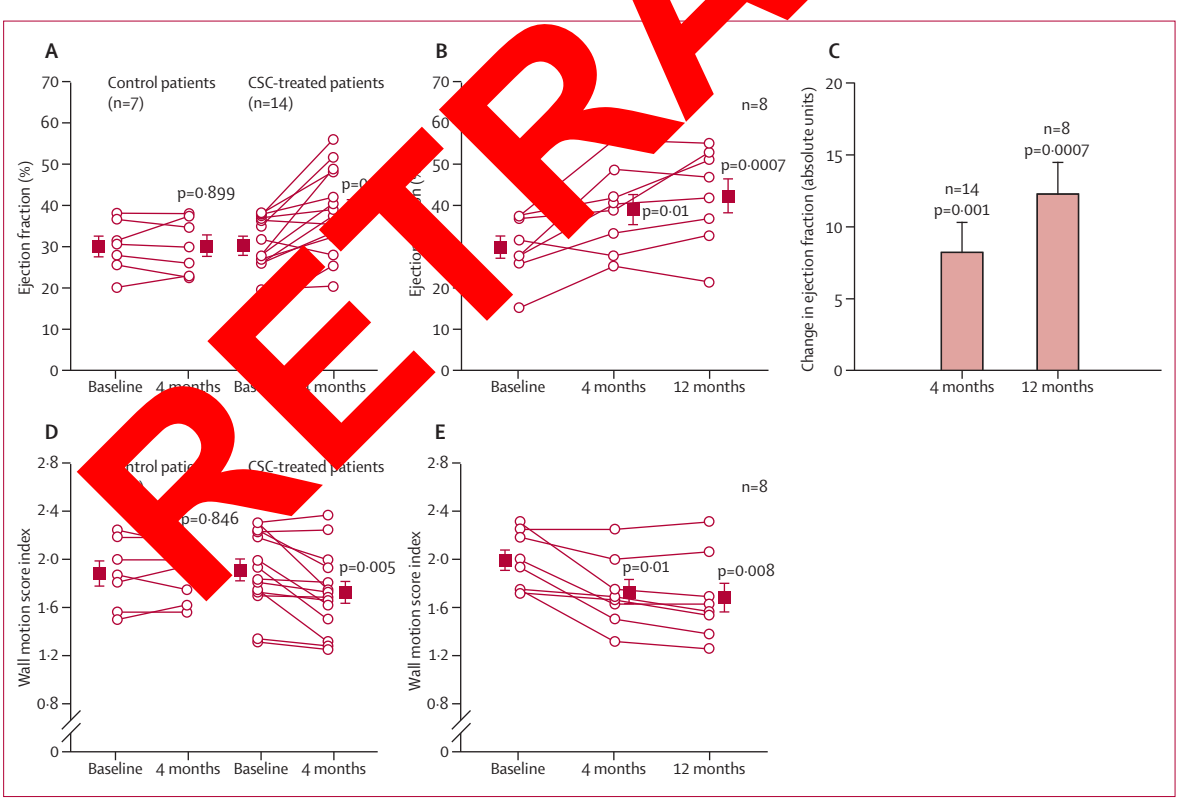

Figure 4: **Echocardiographic analysis of CSC-treated patients and controls**
(A) Left ventricular ejection fraction (measured by use of three-dimensional echocardiography) at 4 months after baseline in control and CSC-treated patients. (B) Ejection fraction at 4 months and 12 months after baseline in 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 fr[om] (3·6) at 4 months to 42·5% (4·1) at 1 year (fi[gure] 4B); alt[hough] the increase from 4 months to 12 [month]s was [not] significant (p=0·159), it sugges[ts] that C[SC] continu[e] to improve LV function beyond [the] first 4 mon[ths]. Th[e] absolute increase in LVEF from [base]line w[as] 3·2 EF [units] (2·0) at 4 months in 14 patient[s] and 12 [LV]EF units (2·1) at 12 months in eight pa[tient]s (f[igure] 4C). An [im]provement in LVEF was noted [in] 12 of 14 p[atient]s a[t] 4 months (figure 4A) and in all [the] patients wi[th] completed follow-up at 1 year (f[igure] 4[C]). By contrast, in the seven control patients with 4 months [of] follow-up, none of these values changed much over th[e] 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 m², 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

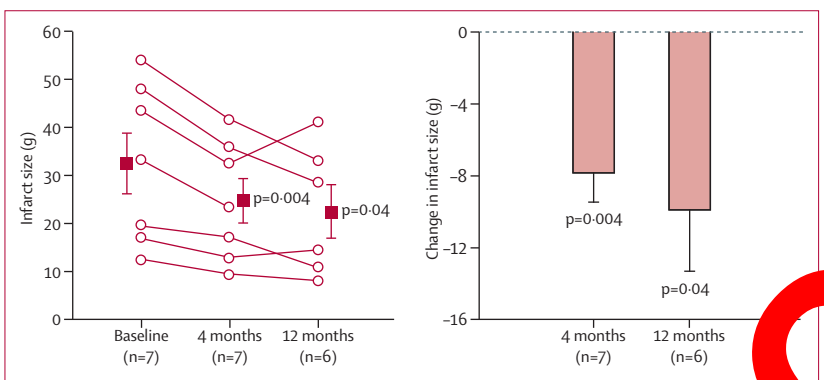

Figure 5: **Infarct size and change in infarct size at 4 months and 12 months after baseline in patients administered cardiac stem cells**

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

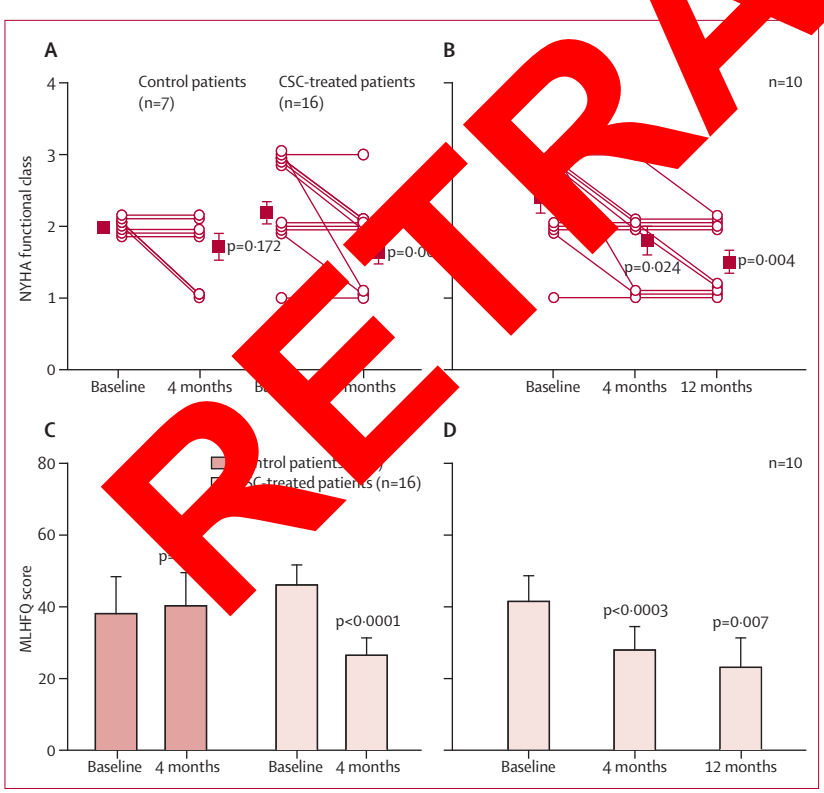

Figure 6: **Functional status and quality of life in control and CSC-treated patients**

(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.

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	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 (immediate 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 treatment), 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, dissection was repaired with a stent and no complications arose during the following 2 years. Information from the SCIPIO trial about the safety and feasibility of CSCs to patients is further described in the appendix 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.[14] These cells are thought to replenish the pool of cardiac myocytes and cardiac vascular cells that die during an organism's lifetime.[5–8] The initial results of SCIPIO are consistent with the salutary effects of CSCs reported in preclinical studies[5,7–11] 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).[4] 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).[2] The optimum dose of CSCs needs to be established. The dose of CSCs used in SCIPIO (1 million) was selected conservatively on the basis of preclinical studies in pigs[8] and the growth characteristics of human 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[7] and senescence occurs when telomeres reach 5–2·0 kbp, a single engrafted CSC could in principle undergo 42 times before irreversible growth arrest generating 4×10¹² cells. We have shown that CSCs can be isolated and expanded from small (about 1 g) endomyocardial biopsies.[14] 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,[28] 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.[29–31] 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.

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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 SCIPIO; webappendix p 6). In four of these studies,[21–24] bone marrow cells were infused intracoronarily (in one study,[24] 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 salubrious effects of CSCs on left ventricular (LV) function and structure in mouse, rat, dog, and pig models of post-myocardial infarction heart failure. SCIPIO differs from all previous trials of cell therapy for heart disease in which non-cardiac cell products (mostly bone-marrow-derived cells[4]) were used. Most of these previous studies were undertaken in patients with acute myocardial infarction; in few studies the investigators seek to treat heart failure caused by chronic ischaemic cardiomyopathy, which was the setting for SCIPIO. 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 in chronic ischaemic cardiomyopathy (webappendix p 6), investigators of the largest studies, which involved coronary infusion was used reported increases in LVEF of 3 EF units (TOPCARE-CHD),[21] 7 EF units (IACT),[22] and 6 EF units (STAR-heart)[23] at 3 months, 5 EF units,[24] 6 EF units (STAR-heart[23]) 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 6 months, and a significant improvement was reported at 6 months and 1 year in one study[27] (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 SCIPIO 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),[28] and thus could not account for the long-term benefits. We emphasise that SCIPIO 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 SCIPIO 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 SCIPIO 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, designed and managed the study with input from the group. PA, DD'A, AL, TH, FS, PG, DC, DGR, and UH designed, undertook, and interpreted all experiments involving CSCs. MFS and GW designed, undertook, and interpreted all echocardiographic studies. AB designed, interpreted, and did all cMRI studies. AQ designed and did interventional procedures. ARC, JHL, ANKS, 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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References

1 Coronary Heart Disease Statistics. London: British Heart Foundation, 2003.
2 Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation* 2011; **123:** e18–e209.
3 Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJV. More malignant than cancer? Five year hospitalization after a first hospitalization with heart failure. *Eur Heart Fail* 2001; **3:** 315–22.
4 Abdel-Latif A, Bolli R, Tleyjeh IM, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch intern Med* 2007; **167:** 989–97.
5 Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 2003; **114:** 763–76.
6 Linke A, Muller P, Nurzynska D, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *Proc Natl Acad Sci USA* 2005; **102:** 8966–71.
7 Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. *Proc Natl Acad Sci USA* 2007; **104:** 14068–73.
8 Bolli R, Jneid H, Tang XL, et al. Intracoronary administration of cardiac stem cells improves cardiac function in pigs with old infarction. *Circulation* 2006; **114** (18 suppl): II_239.
9 Dawn B, Stein AB, Urbanek K, et al. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infracted myocardium, and improve cardiac function. *Proc Natl Acad Sci USA* 2005; **102:** 3766–71.
10 Tang XL, Rokosh G, Sanganalmath SK, et al. Intracoronary administration of cardiac progenitor cells alleviates left ventricular dysfunction in rats with a 30-day-old infarction. *Circulation* 2010; **121:** 293–305.
11 Fischer KM, Cottage CT, Wu W, et al. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. *Circulation* 2009; **120:** 2077–87.
12 Rota M, Padin-Iruegas ME, Misao Y, et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. *Circ Res* 2008; **103:** 107–16.

13 The Criteria Committee of the New York Heart Association. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th edn. Boston, MA: Little, Brown and Co, 1994: 253–56.

14 D'Amario D, Fiorini C, Campbell PM, et al. Functionally competent cardiac stem cells can be isolated from endomyocardial biopsies of patients with advanced cardiomyopathies. *Circ Res* 2011; **108:** 857–61.

15 D'Amario D, Cabral-Da-Silva MC, Zheng H, et al. Insulin-like growth factor-1 receptor identifies a pool of human cardiac stem cells with superior therapeutic potential for myocardial regeneration. *Circ Res* 2011; **108:** 1467–81.

16 Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitiation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989; **2:** 358–67.

17 Kim RJ, Shah DJ, Judd RM. How we perform delayed enhancement imaging. *J Cardiovasc Magn Reson* 2003; **5:** 505–14.

18 Flett AS, Hasleton C, Cook C, et al. Evaluation of techniques for the quantification of myocardial scar of differing etiology using cardiac magnetic resonance. *JACC Cardiovasc Imaging* 2011; **4:** 150–56.

19 Aubert G, Lansdorp PM. Telomeres and aging. *Physiol Rev* 2008; **88:** 557–79.

20 Beausejour CM, Campisi J. Ageing: balancing regeneration and cancer. *Nature* 2006; **443:** 404–05.

21 Assmus B, Honold J, Schachinger V, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 2006; **355:** 1222–32.

22 Strauer BE, Brehm M, Zeus T, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. *J Am Coll Cardiol* 2005; **46:** 1651–58.

23 Strauer BE, Youssef M, Schannwell CM. The acute and long-term effects of intracoronary stem cell transplantation in 191 patients with chronic heart failure: the STAR-heart study. *Eur J Heart Fail* 2010; **12:** 721–29.

24 Hu S, Liu S, Zheng Z, et al. Isolated coronary artery bypass graft combined with bone marrow mononuclear cells delivered through a graft vessel for patients with previous myocardial infarction. chronic heart failure. *J Am Coll Cardiol.* 2011; **57:** 2409–15.

25 Chamuleau SAJ, Vrijsen KR, Rokosh DG, Tang XL, Piek olli R. Cell therapy for ischaemic heart disease: focus on the resident cardiac stem cells. *Neth Heart J* 2009; **17:** 199–20

26 Perin, EC, Silva GV, Henry TD, et al. A random study of transendocardial injection of autologous marrow mononuclear cells and cell function analy ischem eart failure (FOCUS-HF). *Am Heart J* 2011; 078–87.

27 Williams AR, Trachtenberg B, Velaz uez ramyocar l stem cell injection in patients wi hemic myopathy Functional recovery and rever odeling. C 201 **108:** 792–96.

28 Heusch G. Hibernating ar *Physiol Rev* 19 **8:** 1055–85.

29 Vanoverschelde JL, Depr C, Gerbe t al. Time course of functional recovery after coronary arte ass graft surgery in patients with c ventricular isch dysfunction. *Am J Cardiol* **85:** 1432–39.

30 Lorusso R, anna G, Ceconi C, et al. Long-term results of coronary ar bypass graftin ocedure in the presence of left ventricu ysfunction ar bernating myocardium. *Eur J Cardiot Surg* 2001; 37–48.

31 Mintz LJ, Ingels rs GT 2nd, Stinson EB, Adlerman El. ial studies o ntricular function and wall motion after bre ial bypass surgery. *Am J Cardiol* 1980; **45:** 210–16.

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