Online Data Supplement

Methods

Patients

Fifty-five consecutive patients affected by chronic coronary artery disease were included in the study (Figure I in the online-only Data Supplement). Patients underwent elective on-pump CABG for multi-vessel coronary atherosclerosis at the Policlinico A. Gemelli, Catholic University, in Rome. An initial screening was performed prior to surgery to determine the eligibility of patients to participate in the study. The inclusion criteria were: **1.** chronic stable angina in the last 6 months, together with evidence of ischemia at stress test; and **2.** complete surgical revascularization within 7 days. The exclusion criteria were: **1.** evidence of previous myocardial infarct or pathologic Q waves on the 12-leads ECG; **2.** hospitalization for acute coronary syndrome during the last 6 months; **3.** severe valvular heart disease; **4.** coronary artery by-pass graft (CABG) or percutaneous trans-luminal angioplasty in the preceding 12 months; **5.** life expectancy less than 1-year; **6.** presence of malignancies; **7.** psychosocial conditions precluding long-term adherence; **8.** pregnancy; **9.** enrollment in other clinical research trial; **10.** cardiogenic shock; **11.** severe comorbidities; and **12.** patients unable or unwilling to give informed written consent.

Enrolled patients, who met the specified criteria, were subjected to harvesting of the right atrial appendage during on-pump CABG. A follow-up visit was scheduled 12±1 months after CABG. At the time of enrollment and follow-up, patients had complete clinical and physical examination with collection of cardiovascular risk factors, 2D echocardiography, and evaluation of NYHA functional class. Moreover, the number of diseased vessels and the localization and degree of coronary artery stenosis were evaluated together with the risk stratification score and intra- and peri-operative details (Table 1). Blood samples were drawn at the time of surgery and 12±1 months later to measure the level of IGF-1, HGF, VEGF, SCF, G-CSF, and bFGF. The study was

approved by the local ethics committee and conformed to the Declaration of Helsinki on human research.

Clinical Evaluation and Cardiovascular Risk Factors

In each patient, age, sex, common cardiovascular risk factors, therapy at admission and discharge were recorded. The angiographic assessment of coronary artery disease was also performed. Hypercholesterolemia, diabetes mellitus, and hypertension were considered present if diagnosed during hospitalization or if patients were assuming drugs for these conditions prior to admission. Diagnosis of hypercholesterolemia was made when total serum cholesterol concentration was >200 mg/dL. Patients were considered diabetic if fasting glycemia was >126 mg/dL in ≥2 occasions and hypertensive when blood pressure values were >140/90 mmHg in >4 occasions. Smokers had to consume >3 cigarettes per day at admission. A family history of ischemic heart disease involved the documentation of acute coronary syndrome before 60 years of age in at least one first-degree relative.

The New York Heart Association (NYHA) functional class was determined in all patients before and after CABG. Patients in NYHA functional class I had asymptomatic cardiac disease. Patients in NYHA functional class II showed mild symptoms of cardiac disease, which involved modest shortness of breath and/or angina, and minimal limitations in regular activity. Patients in NYHA class III had marked limitations in activity, e.g. walking short distances (20–100 m) and were comfortable only at rest. Patients in NYHA class IV experienced symptoms at rest and had severe limitations in activity. Predicted operative mortality risk was calculated using Euroscore II and logistic Euroscore. Euroscore values for each patient were measured using the interactive online calculator (www.euroscore.org/calc).

Echocardiography

At enrollment and follow-up, patients were evaluated echocardiographically. Transthoracic echocardiograms were performed according to the guidelines of the European Society of Echocardiography by the same experienced operator (G.B.) using

commercially available equipment (TA700, Artida, Toshiba Medical Systems, Japan) with a second harmonic 2.25-MHz probe to optimize endocardial border visualization. All echocardiograms were digitized and re-analyzed off-line by three experienced operators (FG, AM, CS), who were blinded to clinical and laboratory data. Left ventricular (LV) anterior and posterior wall thickness, and LV end-systolic and end-diastolic diameters were measured from the parasternal long axis view and fractional shortening was calculated. LV end-systolic and end-diastolic volumes were computed, and ejection fraction (EF) was calculated from the apical 4- and 2-chamber views using a modified Simpson's biplane method. LV mass was calculated using the Devereux equation. All measurements were obtained on three cardiac cycles and average values derived.[1-3] Inter- and intra-observer agreement for this analysis was 94% and 96%, respectively. Regional wall motion was scored as follows: 1= normal; 2= hypokinesia; 3= akinesia; and 4= dyskinesia.[4] The wall motion score index was calculated as the sum of the score of all segments divided by the total number of segments. Changes in all these parameters were calculated as Δ from baseline to 12±1 months following CABG. The presence of negative LV remodeling (LVR) was claimed when, with respect to baseline, there was an increase ≥20% in LV end-diastolic volume 12±1 months after surgery.[5]

Isolation of Cardiac Stem Cells (CSCs)

The right atrial appendage was harvested and CSCs were isolated as previously described.[6-8] Following removal of the fibrotic and fat tissue, the specimens weighted 25 to 50 mg. Each sample was kept in a sterile container filled with growth medium; the tissue was minced in small pieces, ~0.2-1 mg each. Subsequently, tissue fragments were transferred into a 100 mm culture dish, washed twice with saline, minced, placed in 50 ml tubes and allowed to sediment. The supernatant was discarded and samples were re-suspended in Ham's F12K medium containing 1-2 mg/ml collagenase; enzymatic digestion was performed at 37°C. The supernatant containing the small cell pool was

transferred into a 15 ml tube filled with growth medium, centrifuged and suspended. Undigested pieces were allowed to sediment and the protocol was repeated 2-3 times.

The unfractionated cell population was plated in the presence of growth medium, which was changed twice weekly. Following 2-3 passages, cells were trypsinized, washed with PBS, and centrifuged. The cell pellet was suspended in 300 μl PBS-EDTA containing microbeads conjugated with mouse monoclonal c-kit antibody (CD117 Microbead kit Human, Miltenyi). Following incubation for 20 minutes at 4°C on a 360° swinging rotor, the cell suspension was transferred to columns in the Mini-MACS system. Columns were washed 3 times, the washing solution collected, and the depleted c-kit-negative cells discarded. The column was then detached from the Magnetic System and the plunger applied to the column in order to separate the c-kit-positive cells. These cells were flushed into a 15 ml tube; c-kit-positive cells were centrifuged, re-suspended in growth medium, and plated in culture dishes. Following magnetic separation, c-kit-positive cells were analyzed by flow-cytometry to measure the presence of epitopes specific for myocytes, endothelial cells (ECs), and smooth muscle cells (SMCs).

FACS Analysis

Aliquots of CSCs at P5-P6 were fixed in 4% paraformaldehyde for 15 minutes at room temperature and incubated with antibodies against transcription factors, cytoplasmic proteins, and surface antigens (Table I in the online-only Data Supplement). The presence of epitopes of bone marrow hematopoietic and mesenchymal stem cells was examined together with the expression of myocyte-specific (GATA4, Nkx2.5, MEF2C, α-sarcomeric actin), SMC-specific (GATA6, α-smooth muscle actin) and EC-specific (Ets1, von Willebrand factor) markers. In all cases, CSCs were incubated with the primary antibody for 45 minutes at 37°C. Flow cytometry was performed with FACSAria (Becton Dickinson) or Accuri C6 (Accuri Cytometers) instruments. Cellular debris and aggregates were gated out based on forward and side scatter. Gating on the signal of the nuclear stain DAPI was employed to exclude dead cells and additional artifacts. Isotype-

matched negative controls were utilized to define the threshold for each specific signal and establish the appropriate gate for positive cells.[6-10] Data were analyzed with the instrument software.

Population Doubling Time (PDT)

Following characterization by FACS, CSCs were plated at low density, 100 cells per cm^2, and the number of cells per unit area of the culture dish was measured daily for 5-7 days. PDT was computed by linear regression of log2 values of cell number. Only values in the exponential growth phase were used.[6,8,9]

Telomere Length

Telomere length in CSCs was measured by flow-FISH.[7,11,12] Calibration of the flow cytometer, cell fixation, staining protocol, and normalization were performed utilizing mouse lymphoma cells with known telomere length. Approximately 5 x 10^5 CSCs and mouse lymphoma cells with long telomeres (L5178Y-R) were washed in hybridization buffer and re-suspended in hybridization solution containing formamide and 0.3 μg/ml FITC-conjugated with a telomere specific PNA probe. Control samples were incubated in hybridization solution in the absence of the PNA probe. Lymphoma cells were distinguished from CSCs by immunolabeling with CD45 antibody. DNA content was established by propidium iodide staining. Based on DNA content, cells were gated at G0/G1 and the fluorescence intensity of telomeres was calculated. Measurements were performed by FACSAria.

Telomerase Activity

The catalytic activity of telomerase in CSCs was assessed by quantitative PCR.[7-10,13] CSCs were homogenized in CHAPS buffer and centrifuged at 4°C. Two different protein concentrations, 0.5 and 1 μg, were employed for the assay. CSC extracts were incubated in a solution containing reverse transcriptase reaction mix and Taq polymerase (TRAPEZE RT Telomerase Detection Kit, Chemicon) at 30°C for 30 minutes. HeLa cells were used as positive control and serial dilutions of control template TSR8 were employed for quantification. CHAPS buffer in the absence of protein lysates was used as

negative control. PCR cycling conditions were as follows: 1 cycle of 95°C for 2.0 minutes; 40 cycles of 94°C for 15 seconds, and 59°C for 60 seconds. Data were collected at the 59°C stage of each cycle.

Western Blotting and Immunoprecipitation

Protein lysates of cultured CSCs were obtained using CHAPS buffer (Millipore) and protease inhibitors (Complete tablets, Roche). Equivalents of 5-10 μg of proteins were separated on 4-20% SDS-PAGE, transferred onto PVDF membranes (Bio-Rad) and subjected to Western blotting with rabbit polyclonal anti-Akt (Cell Signaling) and rabbit polyclonal anti-phospho-Akt^{Ser473} (Cell Signaling) diluted 1:1500 in TBST containing 5% BSA overnight at 4°C.[9,14] Proteins were detected by chemiluminescence (ECL Plus, Thermopierce). Optical density was measured and the quantification was performed as ratio of phospho-Akt to total Akt. Loading conditions were determined by the expression of GAPDH (Cell Signaling).

Equivalents of 50 μg of protein extracts were incubated with rabbit polyclonal anti-telomerase antibody (Abcam) overnight at 4°C and subsequently were exposed to magnetic protein G (Millipore) for 1 hour at room temperature.[15-17] Immunoprecipitated proteins were eluted by adding 2X loading buffer and heating at 95°C for 5 minutes. Proteins were separated on 4-20% SDS-PAGE and transferred onto a PVDF membrane. Blots were incubated overnight at 4°C with rabbit polyclonal anti phospho-telomerase at Ser824 (Abcam). Optical density was measured and the quantification was performed as ratio of phospho-telomerase to total telomerase.

Akt Kinase Assay

This assay was performed according to the manufacturer's protocol (Cell Signaling). Protein lysates were immunoprecipitated using phospho-Akt antibody conjugated with Sepharose beads overnight at 4°C. After washing, GSK-3α/β substrate was added and the enzymatic reaction was carried on for 30 minutes at 30°C. The reaction was stopped by adding loading buffer and heating at 95°C for 5 minutes. Following centrifugation, the supernatant was loaded on 4-20% SDS-PAGE and transferred onto a PVDF membrane.

Blots were incubated overnight at 4°C with rabbit polyclonal anti phospho-GSK-3α/β. The detection of a band of the correct molecular weight was indicative of Akt kinase activity in the sample.[18]

Growth Factors

To obtain an accurate, efficient, and quantitative determination of the concentration of multiple human growth factors in the patient's serum, a multiplex microarray technology based on two-site sandwich ELISA was employed. Capture antibodies specific for target growth factors are pre-spotted into each well of a 96 well microplate so that the growth factors present in the samples are bound by the immobilized antibodies. This approach allowed us to detect simultaneously 6 growth factors in the same sample of serum. Standards and samples were added to the wells. Following washing of the unbound proteins, biotinylated detection antibodies were used. Streptavidin-HRP and, subsequently, chemiluminiscent substrate reagents were added to the wells. A signal proportional to the amount of each growth factor bound to its specific antibody was produced. Plates were read using a digital camera imaging system, and pixel intensity was measured using an analytical software package. A standard curve was created for each analyte by plotting the median pixel intensity for each standard on the y-axis and the concentration of the standard on the x-axis. Data were linearized by plotting the log of the concentrations versus the log of the pixel intensity and the best fit line was determined by regression analysis and corrected for the dilution factor.[19]

Statistical Analysis

Continuous variables were expressed as mean±SD, while dichotomous variables are shown as percentages. Unpaired T-test or Mann Whitney U-test was used for comparison between two groups. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Linear regression analysis was performed to correlate the characteristics of CSCs in vitro with the indices of LVR: in each case the best-fit regression line is showed together with the 95% confidence interval, and the P and R^2 values. P values less than 0.05 were considered significant.

Multivariate logistic regression analysis was performed in order to identify independent predictor of LVR. Variables showing a p value less than 0.05 at univariate analysis were included in the models. Beta value and 95% confidence interval have been reported.

Receiver Operating Characteristic (ROC) curve was performed to determine the cellular biomarker or growth factor level that best predicted negative LVR, and to assess the best cut-off value. The Youden index was introduced to evaluate the sensitivity and specificity of each variable. Statistical comparisons were performed using SPSS 20.0 (SPSS, Inc Chicago, Illinois); however, ROC analysis was done using MedCalc (MedCalc, Mariakerke, Belgium).[20,21] The magnitude of sampled cells is listed in Table II in the online-only Data Supplement.

References

1. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise J, Solomon S, Spencer KT, St John Sutton M, Stewart W. Recommendations for chamber quantification. *Eur J Echocardiogr*. 2006; 7:79-108.

2. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr*. 1989; 2:358-367.

3. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and

Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005; 18:1440-1463.

4. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS; American Heart Association Writing Group on Myocardial Segmentation and Registration for Cardiac Imaging. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. *Circulation*. 2002; 105:539-542

5. Bolognese L, Cerisano G, Buonamici P, Santini A, Santoro GM, Antoniucci D, Fazzini PF. Influence of infarct-zone viability on left ventricular remodeling after acute myocardial infarction. *Circulation.* 1997; 96:3353-3359.

6. Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De Angelis A, Yasuzawa-Amano S, Trofimova I, Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek K, Michler RE, Bolli R, Kajstura J, Leri A, Anversa P. Human cardiac stem cells. *Proc Natl Acad Sci USA.* 2007; 104:14068-14073.

7. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet.* 2011; 378:1847-1857.

8. Kajstura J, Bai Y, Cappetta D, Kim J, Arranto C, Sanada F, D'Amario D, Matsuda A, Bardelli S, Ferreira-Martins J, Hosoda T, Leri A, Rota M, Loscalzo J, Anversa P. Tracking chromatid segregation to identify human cardiac stem cells that regenerate extensively the infarcted myocardium. *Circ Res.* 2012; 111:894-906.

9. D'Amario D, Cabral-Da-Silva MC, Zheng H, Fiorini C, Goichberg P, Steadman E, Ferreira-Martins J, Sanada F, Piccoli M, Cappetta D, D'Alessandro DA, Michler RE, Hosoda T, Anastasia L, Rota M, Leri A, Anversa P, Kajstura J. Insulin-like growth factor-1 receptor identifies a pool of human cardiac stem cells with superior therapeutic potential for myocardial regeneration. *Circ Res.* 2011; 108:1467-1481.

10. D'Amario D, Fiorini C, Campbell PM, Goichberg P, Sanada F, Zheng H, Hosoda T, Rota M, Connell JM, Gallegos RP, Welt FG, Givertz MM, Mitchell RN, Leri A, Kajstura J, Pfeffer MA, Anversa P. Functionally competent cardiac stem cells can be isolated from endomyocardial biopsies of patients with advanced cardiomyopathies. *Circ Res.* 2011; 108:857-861.

11. Canela A, Vera E, Klatt P, Blasco MA. High-throughput telomere length quantification by FISH and its application to human population studies. *Proc Natl Acad Sci USA.* 2007; 104:5300-5305.

12. Baerlocher GM, Vulto I, de Jong G, Lansdorp PM. Flow cytometry and FISH to measure the average length of telomeres (flow FISH). *Nat Protoc.* 2006; 1:2365-2376.

13. Herbert BS, Hochreiter AE, Wright WE, Shay JW. Non radioactive detection of telomerase activity using the telomeric repeat amplification protocol. *Nature Protocols*. 2006; 1:1583-1599.

14. Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, Zias E, Walsh K, Rosenzweig A, Sussman MA, Urbanek K, Nadal-Ginard B, Kajstura J, Anversa P, Leri A. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ Res.* 2004; 94:514-524.

15. Leri A, Barlucchi L, Limana F, Deptala A, Darzynkiewicz Z, Hintze TH, Kajstura J, Nadal-Ginard B, Anversa P. Telomerase expression and activity are coupled with myocyte proliferation and preservation of telomeric length in the failing heart. *Proc Natl Acad Sci USA.* 2001; 98:8626-8631.

16. Boni A, Urbanek K, Nascimbene A, Hosoda T, Zheng H, Delucchi F, Amano K, Gonzalez A, Vitale S, Ojaimi C, Rizzi R, Bolli R, Yutzey KE, Rota M, Kajstura J, Anversa P, Leri A. Notch1 regulates the fate of cardiac progenitor cells. *Proc Natl Acad Sci USA.* 2008;105: 15529-15534.

17. Goichberg P, Kannappan R, Cimini M, Bai Y, Sanada F, Sorrentino A, Kajstura J, Rota M, Anversa P, Leri A. Age-associated defects in EphA2 signaling impair the migration of human cardiac stem cells. *Circulation.* Epub ahead of print, 2013.

18. Thimmaiah KN, Easton J, Huang S, Veverka KA, Germain GS, Harwood FC, Houghton PJ. Insulin-like growth factor I-mediated protection from rapamycin-induced apoptosis is independent of Ras-Erk1-Erk2 and phosphatidylinositol 3'-kinase-Akt signaling pathways. *Cancer Res*. 2003; 63:364-74.

19. Fitzgerald JP, Nayak B, Shanmugasundaram K, Friedrichs W, Sudarshan S, Eid AA, DeNapoli T, Parekh DJ, Gorin Y, Block K. Nox4 mediates renal cell carcinoma cell invasion through hypoxia-induced interleukin 6- and 8- production *PLoS One*. 2012; 7:e30712.

20. Berenson ML, Levine DM, Rindskopf D. *Applied Statistics*. 1988. Englewood Cliffs, NJ.

21. Fawcett T. An introduction to ROC analysis. *Pattern Recognition Letters*. 2006; 27:861-874

Table I. List of Antibodies

Epitope	Manufacturer	Host Animal	Labeling
Surface Markers			
c-kit	DAKO	rabbit	direct, indirect, F, T
CD34	BD Pharmigen	mouse	direct, PE
CD45	BD Pharmigen	mouse	direct, APC
Lineage Markers	BD Pharmigen	mouse	indirect, Cy5
CD90	BD Pharmigen	mouse	direct, PE
CD105	BD Pharmigen	mouse	direct, PE
Transcription Factors			
GATA4	Santa Cruz	rabbit	direct, indirect, F, T
Nkx2.5	Santa Cruz	goat	direct, indirect, F, T
MEF2C	Santa Cruz	goat	indirect, F, T
Ets1	BD Pharmingen	rabbit	indirect F, T
GATA6	Santa Cruz	rabbit	indirect, F, T
Cytoplasmic Proteins			
α-sarcomeric actin	Sigma	mouse	indirect, F, T
von Willebrand factor	DAKO	rabbit	indirect, F, T, Cy5
α-smooth muscle actin	Sigma	mouse	indirect, F, T, Cy5
Other Epitopes			
BrdU	Roche	mouse	indirect, F,Cy5

F: fluorescein isothiocyanate; T: tetramethyl rhodamine isothiocyanate; Cy5: cyanine 5.

Table II. Magnitude of Sampling

Parameter	n value	Aggregate sample size	Sample size (mean±SD)
FACS Analysis			
c-kit	38	675,336 [(1)]	13,766 ± 5,795
CD34	38	658,510 [(1)]	15,851 ± 3,424
CD45	38	656,686 [(1)]	15,668 ± 1,761
CD133	38	644,030 [(1)]	14,403 ± 1,641
Lineage markers	38	661,068 [(1)]	16,108 ± 2,393
CD90	38	654,563 [(1)]	15,456 ± 2,510
CD105	38	655,693 [(1)]	15,563 ± 1,665
Growth and Death			
PDT	38	49,875 [(2)]	1,012 ± 39
BrdU	38	49,032 [(2)]	1,452 ± 349
TdT	38	47,598 [(2)]	1,380 ± 229
Telomere Length	38	223,088 [(1)]	6,154 ± 1307
Telomerase Activity	38	N/A	N/A
Lineage Commitment			
GATA4	38	336,972 [(1)]	7,121 ± 2,775
Nkx2.5	38	424,753 [(1)]	8,594 ± 2,104
MEF2C	38	439,668 [(1)]	9,958 ± 4,122
α-SA	38	394,177 [(1)]	7,397 ± 3,539
GATA6	38	387,377 [(1)]	8,447 ± 4,248
α-SMA	38	387,014 [(1)]	8,376 ± 3,841
CD31	38	436,439 [(1)]	5,804 ± 2,734
vWf	38	432,858 [(1)]	9,107 ± 4,458

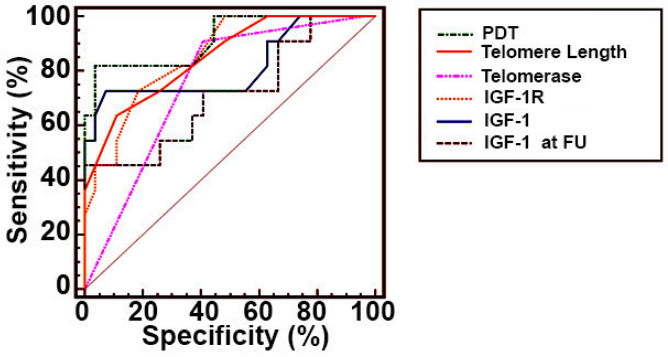
[(1)] Number of cells analyzed by FACS; [(2)] Number of cells examined by confocal microscopy.

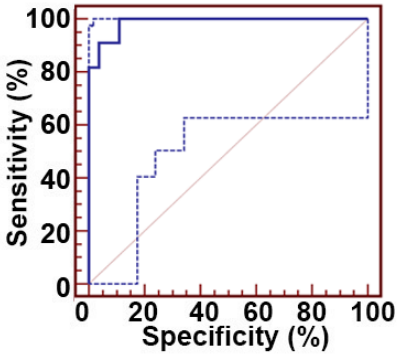
Table III. Predictors of LV Remodeling

PDT	26.0	24.8-28.5	<0.0001
IGF-1R positive CSC (%)	35.5	31.2-39.6	<0.0001
Telomere Length (kbp)	7.0	6.8-7.2	<0.0001
Telomerase Act. (n° copies 1x 10^5)	1.6	1.4-1.8	<0.0001
IGF-1 at baseline (ng/ml)	35.4	19.2-51.6	<0.0001
IGF-1 at follow-Up	70.6	47.4-93.7	<0.0001
HGF at baseline (pg/ml)	884.6	811.6-1257.5	=0.0921
VEGF at baseline (pg/ml)	253.9	231.4-397.6	=0.0873

Table IV. ROC Analysis.

	AUC	p	CI 95%	Cut Off	OR	Sensitivity	Specificity
PDT	0.92	<0.001	0.78- 0.98	>26.8	25.9	81.8	96.3
Telomere length	0.85	< 0.001	0.70- 0.94	≤7	31.2	90.9	59.3
Telomerase	0.75	0.002	0.59- 0.88	≤1.6	14	63.6	88.9
IGF1-R	0.86	<0.001	0.71- 0.95	≤39	11.7	72.7	81.5
IGF1	0.81	<0.001	0.66- 0.92	≤52.7	69.3	72.7	96.3
IGF1 at follow-up	0.71	0.01	0.54- 0.85	≤81.8	0.18	45.4	100





	AUC	p	CI 95%
Multivariable logistic regression model	0.98	<0.001	0.01-0.99

Legend to Figures

Figure I. Patient population and study design.

Figure II. Phenotype of CSCs. **A,** Bivariate distribution of c-kit, epitopes of hematopoietic stem cells (CD34, CD45), mast cells (CD45), mesenchymal stromal cells (CD90, CD105), and cocktail of bone marrow cell lineages. **B,** Bivariate distribution of c-kit and epitopes of cardiomyocytes (GATA4, Nkx2.5, MEF2C, α-SA), ECs (Ets1, vWf) and SMCs (GATA6, α-SMA).

Figure III. CSCs and anatomical indices of LVR. Correlations between c-kit expression in CSCs (showed in percentage) and indices of cardiac shape (Δ end-systolic volume and Δ end-diastolic volume) and function (Δ ejection fraction). These relationships are shown by linear regression.

Figure IV. Growth reserve of CSCs and anatomical indices of LVR. Analysis of PDT, telomere length, telomerase activity and fraction of IGF-1R-postive ($IGF\text{-}1R^{pos}$) CSCs in patients divided in tertiles according to the variation (Δ) of parameters of cardiac shape (end-systolic volume and end-diastolic volume) and function (ejection fraction). Data are shown as mean±SD. ESV, end-systolic volume; EDV, end-diastolic volume; EF, left ventricular ejection fraction. *Indicates $P<0.05$ vs. I tertile values; **Indicates $P<0.05$ vs. II tertile values.

Figure V. Ischemic burden and anatomical indices of LVR. Correlations between pre-operative burden of ischemia, expressed as double product values at 1 mm ST-segment depression during stress test and indices of cardiac shape (end-systolic volume and end-diastolic volume) and function (ejection fraction). These relationships are shown by linear regression.

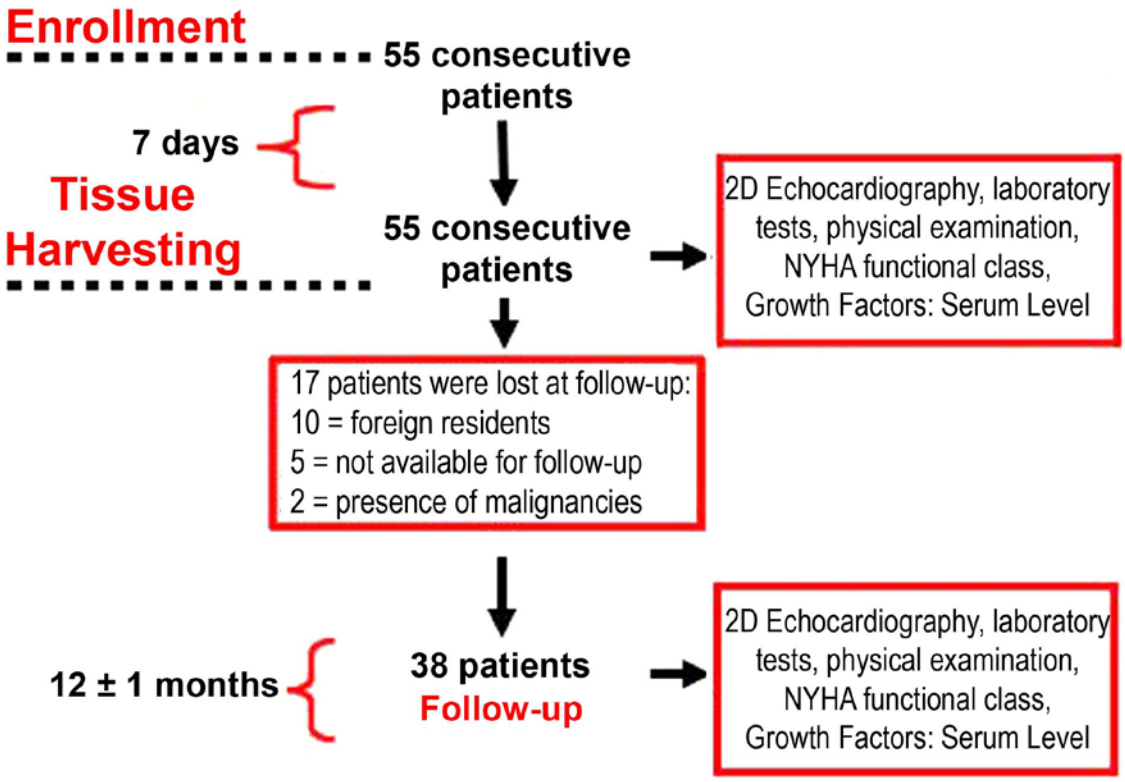

Enrollment
55 consecutive patients
7 days
Tissue Harvesting
55 consecutive patients
2D Echocardiography, laboratory tests, physical examination, NYHA functional class, Growth Factors: Serum Level
17 patients were lost at follow-up:
10 = foreign residents
5 = not available for follow-up
2 = presence of malignancies
12 ± 1 months
38 patients
Follow-up
2D Echocardiography, laboratory tests, physical examination, NYHA functional class, Growth Factors: Serum Level

Figure I

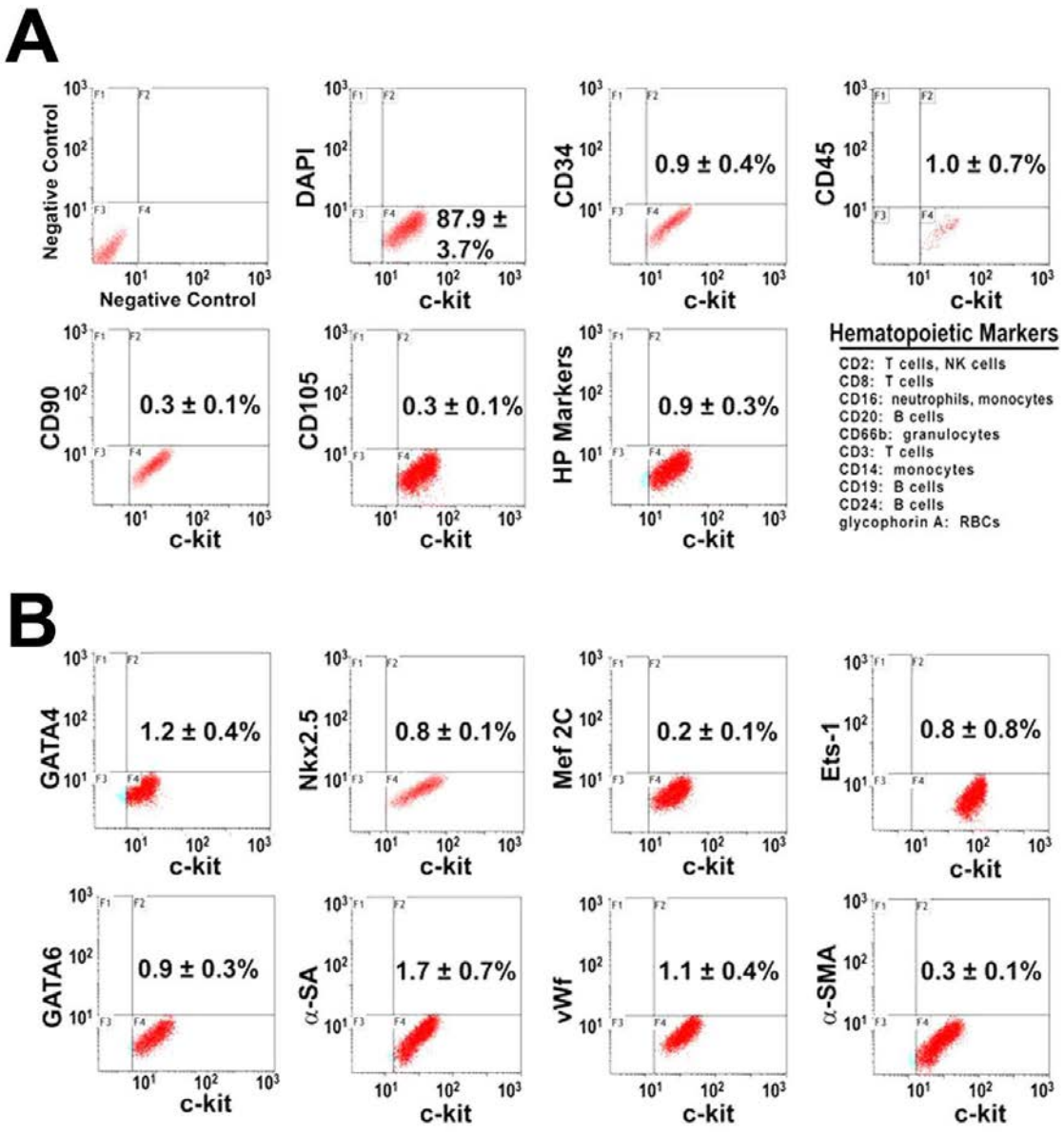
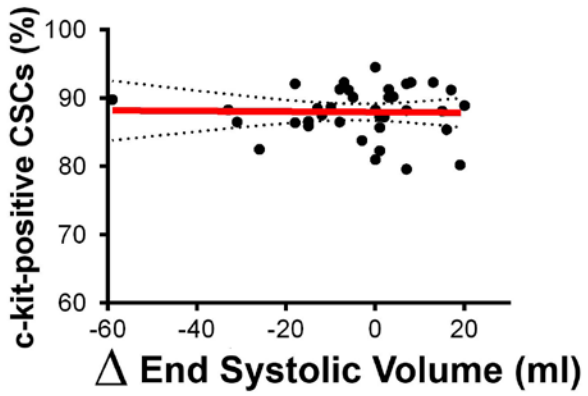
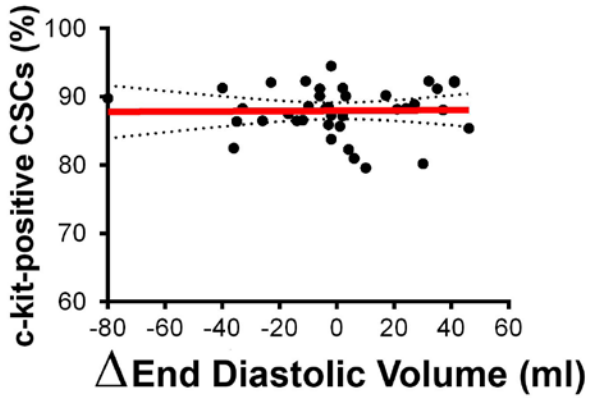

A
Negative Control
Negative Control
DAPI
87.9 ± 3.7%
CD34
0.9 ± 0.4%
CD45
1.0 ± 0.7%
CD90
0.3 ± 0.1%
CD105
0.3 ± 0.1%
HP Markers
0.9 ± 0.3%
c-kit
Hematopoietic Markers
CD2: T cells, NK cells
CD8: T cells
CD16: neutrophils, monocytes
CD20: B cells
CD66b: granulocytes
CD3: T cells
CD14: monocytes
CD19: B cells
CD24: B cells
glycophorin A: RBCs
B
GATA4
1.2 ± 0.4%
Nkx2.5
0.8 ± 0.1%
Mef 2C
0.2 ± 0.1%
Ets-1
0.8 ± 0.8%
GATA6
0.9 ± 0.3%
α-SA
1.7 ± 0.7%
vWf
1.1 ± 0.4%
α-SMA
0.3 ± 0.1%

Figure II A-B

c-kit-positive CSCs and Left Ventricular Remodeling





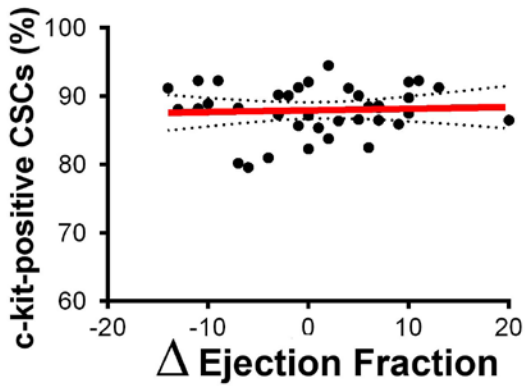


Figure III

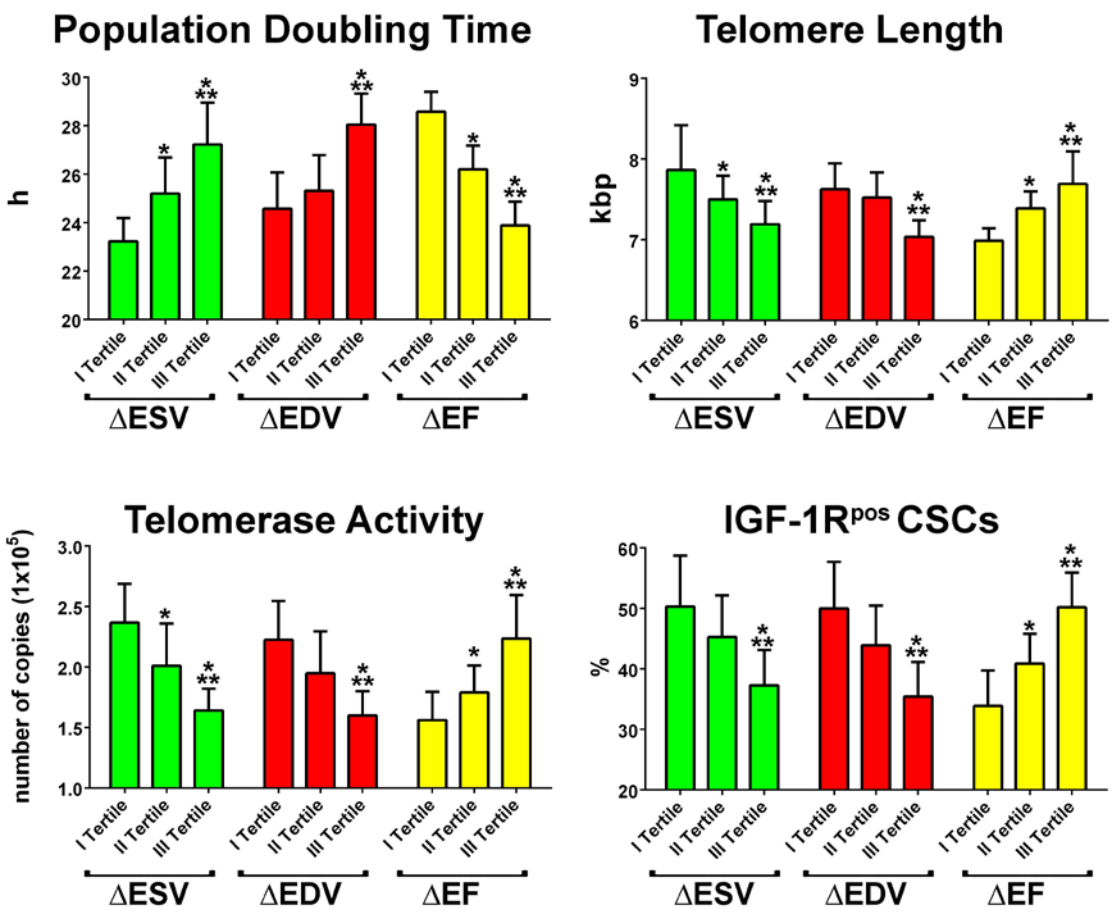
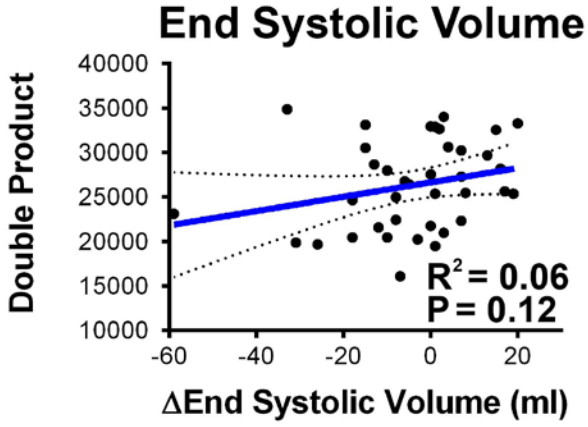
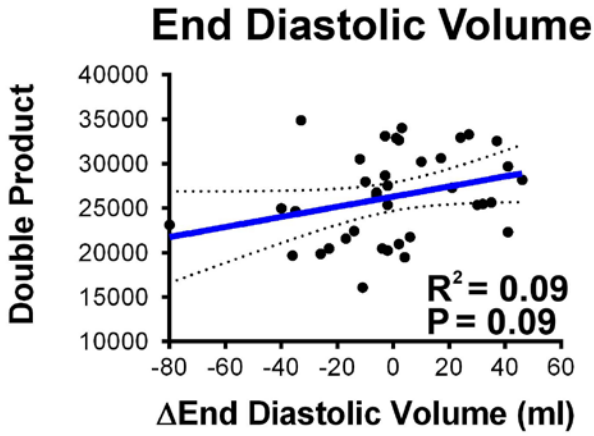

Population Doubling Time
h
30
28
26
24
22
20
I Tertile
II Tertile
III Tertile
ΔESV
ΔEDV
ΔEF
Telomere Length
kbp
9
8
7
6
Telomerase Activity
number of copies (1x10^5)
3.0
2.5
2.0
1.5
1.0
IGF-1R^{pos} CSCs
%
60
50
40
30
20

Figure IV

Double Product at 1 mm ST-segment depression during baseline stress test





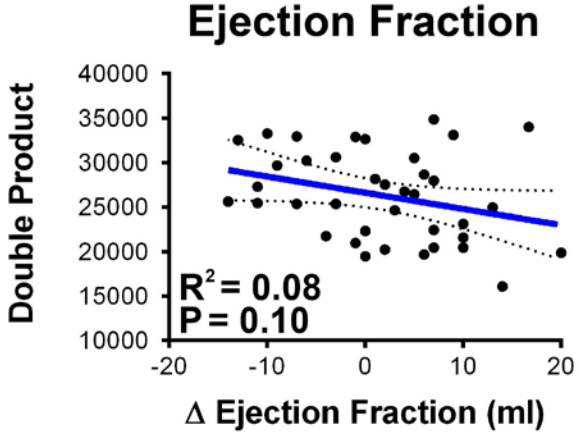


Figure V