SUPPLEMENTARY MATERIAL ONLINE.

(Expanded Methods and Results)

Methods

List of exclusion criteria

Main exclusion criteria were: age >75 years; previous myocardial infarction; patient instability after myocardial infarction; cardiac disease except ischemic heart disease; multivessel coronary artery disease; need for coronary revascularization in the future; pulmonary edema and cardiogenic shock; advanced renal or hepatic failure; non-cardiac disease adversely affecting prognosis; coagulopathy, thrombocytopenia, and leukopenia.

Harvest and transfer of bone marrow cells (BMCs):

Bone marrow harvest and cell preparation

A total of 50 mL of bone marrow was aspirated into heparin-treated syringes from the iliac crest under local anesthesia. The aspirate was shipped at room temperature to the cell-processing laboratory of each local center. Progenitor cells were isolated and enriched using lymphocyte preparation medium centrifugation procedures (Ficoll, Eurobio). The cell suspension consisted of a heterogeneous cell population including hematopoietic, endothelial, and other progenitor cells, as well as mononuclear cells. A single syringe of 100 x 10^6 BMCs was prepared in 10 ml 4% human albumin; all safety analyses were performed before release of the final cell therapy product in accordance with the French regulations.

Flow cytometry analysis of BMCs

Cell therapy product (adjusted to 5000 cells/μL) was analyzed by flow cytometry. For the identification of cell populations, the following conjugated anti-humans antibodies were used: anti-CD45 (PC7-labeled; Beckman Coulter), anti-CD34 (APC-labeled; Beckman Coulter), anti-CD133 (PE-labeled; Miltenyi), anti-KDR (PE-labeled; R&D System), and anti-CXCR4 (PE-labeled; BD Pharmingen). Acquisition was performed on a FACS Calibur (Becton Dickinson) and 250 000 CD45-positive events were recorded. Analysis was performed using CellQuest Software (BD Biosciences) by two independent investigators, according to the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines.[1]

Colony-forming unit assay

BMCs (1 x 10^5/dish) were seeded in duplicates in methylcellulose plates (Methocult GF H4534; StemCell). Plates were studied by phase-contrast microscopy, and granulocyte-macrophage colony-forming units (CFU-GM; colonies with > 40 cells) were counted by two independent investigators after 14 days incubation at 37°C.

Cell administration

BMCs were infused using a stop-flow technique through an over-the-wire balloon catheter positioned within the segment containing the stent, as described previously.[2] Briefly, after undergoing arterial puncture, all patients received 5 000−10 000 IU heparin. The reconstituted final cell preparation was injected in three fractions over 2–3 min using a perfusion catheter (Maverick, Boston Scientific) during three low-pressure stop-flow inflations in the stent to allow the BMCs maximum contact time with the microcirculation of the infarct-related artery. The procedure was repeated three times with a 3-min reperfusion period between injections to reduce the likelihood of ischemia to a minimum. After completion of cell transfer, contrast medium was injected into the infarct-related artery to determine vessel patency.

Follow-up

All patients were treated with aspirin and clopidogrel for at least 4 weeks (300 mg loading dose then 75 mg daily after PCI), an angiotensin-converting enzyme (ACE) inhibitor, a β-blocker, and a statin, unless these agents were contraindicated. Three months after discharge, patients had follow-up examinations to assess their clinical status and to review their current medication. When necessary, dosages of ACE-inhibitors, β-blockers, and statins were adjusted in accordance with current guidelines.[3]

RNA and SPECT

All patients underwent a rest myocardial perfusion SPECT 4 days and 3 months after myocardial infarction, 4 h after an intravenous rest injection of Tl-201 (1.5 MBq/kg). SPECT was acquired using a dual head camera and followed by the acquisition of planar equilibrium radionuclide angiography in left anterior oblique 45° best septal view according to an in vivo red blood cell labeling technique. All raw data were stored in dicom 3 format and were transferred to the Nuclear Cardiology core laboratory for further analysis using a dedicated workstation (Mirage workstation; Segami Corp). Left ventricular ejection fraction was calculated using a count-based method after correction for background noise with automated software. SPECT data were reconstructed by filtered backprojection using a Butterworth filter (order 5, cut-off frequency 0.35; Nyquist). After reconstruction and setting image reorientation and areas of analysis (determination of LV base and apex), myocardial perfusion quantitative analysis was performed using a commercially available automated software (CardioGam, Segami Corp.). Analyses were performed by two experienced observers blinded to any clinical data. Cohen's kappa coefficient was 0.99 for the intra-observer agreement of viability measurement and 0.97 for the inter-observer agreement. Infarct size was defined as the percentage of pixels of the left ventricle with a tracer uptake

≤50% of the maximal uptake. For comparisons with MRI results, non-viable and equivocal segments were analyzed together as non-viable segments. Comparisons were performed by analysis of the total number of segments per group or on a segment-to-segment comparison.

Cardiac MRI

Protocol for images acquisition

Cardiac MRI was performed in the supine position on 1.5 Tesla MR scanners with phase array cardiac coils and prospective ECG gating during repeated breath-holds. Cine-SSFP was performed in three axes (short axis, SA; long axis, LA; four chambers, 4CH) (TR/TE: 3.2/1.6; flip angle 70-90°; matrix 160 x 256; FOV: 37 x 37 cm; slice thickness 5 mm). Ten min after injection of 0.2 mmol/kg of Gd-DTPA (Dotarem, Guerbet, France) contrasted enhanced images were acquired in SA and 4CH view with segmented three-dimensional inversion-recovery gradient echo sequences and linear k-space order. The inversion time selected to optimally null the myocardium was validated by visual inspection using a TI-scouting sequence to determine the best inversion time. Contrast-enhancement MRI was used to assess myocardial infarct size after acute myocardial infarction.[4]

Assessment of cardiac function, scar extent and microvascular obstruction

All cardiac MR results were analyzed by independent radiologists belonging to a core laboratory. Images were analyzed using a dedicated post-processing software (MASS; Medis, Leiden, The Netherlands).

LV function (end diastolic volume, end systolic volume, and ejection fraction) was calculated on short axis views acquired with SSFP sequences. Regional contractility was studied in 17 segments according to American Heart Association standardized myocardial segmentation.[5] For each segment contractility was scored as 1 (normal), 2 (hypokinetic) and 3 (akinetic). The wall motion score index (WMSI) was derived by dividing the total wall motion score by the

number of segments. Infarction-related regional WMSI was derived by dividing the wall motion score of segments with impaired contractility by the number of these segments in each patient. The same segments were taken into account for follow-up regional WMSI assessment. Delayed contrast enhancement was defined as an area fulfilling the following criteria: 1- a signal intensity (SI) value >2 standard deviations above the SI of normal myocardium, 2- presence in the same myocardial segment in two different planes. A quantitative analysis of scar extent was performed by MASS software, expressed as absolute value of hyper enhanced myocardium in grams, and relative value as % of total LV mass (hyper enhanced myocardium/LV mass in grams). Regional transmurality of hypersignal into the myocardium was then classified as null (no hypersignal, grade 0), <25% (sub-endomyocardium, grade 1), between 25 and 75 (grade 2), and > 75% (transmural, grade 3) for statistical analysis. The presence, location, and pattern of each abnormality were independently assessed by two expert radiologists blinded to the clinical findings. In cases of discrepancy, a consensus was reached by discussion.

Presence of microvascular obstruction was defined as hypoenhanced areas (dark) surrounded by areas of hyperenhancement (bright).

For comparisons with SPECT results, grade 0 and grade 1 segments were analyzed together as "viable" segments, and grade 2 and grade 3 segments were analyzed together as "non-viable" segments.

Coronary angiography

Restenosis was quantified with a computer-based system (Medcom System LTD, Telemedicine Technology) by investigators unaware of treatment assignment. Binary restenosis was defined as stenosis of >50% of the luminal diameter analyzed at 3 months.

Power analysis and statistical methods

Our objective was to demonstrate an improvement of at least 2 non-viable segments between both groups (0.5 vs. 2.5). Based on the literature we estimated the standard deviation equal to 2.7.[6] In these conditions, the number of patients required for alpha equal to 5% in bilateral situation and beta equal to 10%, was 40 per group. 101 patients were included in order to maintain the power for a subgroup analysis in case of a significant interaction with a major prognosis factor. Interaction tests between arms and prognostic factors were used to assess the homogeneity effect of each factor, with respect to the proof of model homoscedasticity.[7]

Results

Secondary endpoints

Left ventricular ejection fraction (LVEF) results were confirmed by echocardiography (LVEF from baseline to 3 months: 39.8 ± 7.0 to 41.5 ± 8.8% in the control group vs. 38.1 ± 7.9 to 39.1 ± 10.2% in the BMC group, p = 0.7) and MRI measurements (EF from baseline to 3 months: 38.7 ± 9.2 to 39.1 ± 10.2% in the control group vs. 37 ± 9.8 to 38.9 ± 9.7% in the BMC group, p = 0.4). Left ventricular remodeling assessed by MRI was identical in both groups, with no significant differences in indexed end-diastolic (EDV) and end-systolic volume (ESV) (control group: (EDV/ESV) 56.9 ± 13.1/34.6 ± 10.0 mL/m^2 at baseline to 66.1 ± 18.4/40.0 ± 16.4 mL/m^2 at 3 months; BMC group 57.9 ± 15.6/36.6 ± 12.9 mL/m^2 at baseline to 69.4 ± 23.0/43.7 ± 20.5 mL/m^2 at 3 months, p = 0.91/0.66).

There was no significant difference in changes in infarct size measured by late enhancement MRI between the groups at 3 months (27.7 ± 9.5% versus 30.9 ± 13.9%, p = 0.59). Using a segment-to-segment comparison between day 0 and 3 months, only 32 non-viable segments became viable (2.6% of the total segment number), and 93 improved their contractility (7.7% of the total segment number). There was no agreement between improvement in late

enhancement and improvement in contractility assessed by MRI (kappa = 0.01). Interestingly, there was an increase in contractility at 3 months in 57/183 viable and non-contractile segments at day 0, suggesting that viability per se was not a determinant of mechanical function.

At baseline, the total number of non-viable segments on SPECT was higher than the total number of non-viable segments on MRI (46.9% (718/1530) non-viable segments on SPECT *versus* 40.5 % (493/1216) non-viable segments on MRI). The association between SPECT and MRI viability on a segment-to-segment analysis was statistically significant, with 81% viable segments on SPECT being viable on MRI (chi-square test, $p < 0.0001$). At 3 months the difference was no more significant (38% (582/1530) non-viable segments on SPECT *versus* 39.4 % (480/1216) non-viable segments on MRI, $p = 0.46$) with 76% viable segments on SPECT being viable on MRI, because of the higher increase in viability in SPECT.

Supplemental References

1. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J Hematother* 1996;**5**:213-226.

2. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 2002;**106**:3009-3017.

3. Antman EM, Hand M, Armstrong PW, Bates ER, Green LA, Halasyamani LK, Hochman JS, Krumholz HM, Lamas GA, Mullany CJ, Pearle DL, Sloan MA, Smith SC, Jr., Anbe DT, Kushner FG, Ornato JP, Jacobs AK, Adams CD, Anderson JL, Buller CE, Creager MA, Ettinger SM, Halperin JL, Hunt SA, Lytle BW, Nishimura R,

Page RL, Riegel B, Tarkington LG, Yancy CW. 2007 Focused Update of the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: developed in collaboration With the Canadian Cardiovascular Society endorsed by the American Academy of Family Physicians: 2007 Writing Group to Review New Evidence and Update the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction, Writing on Behalf of the 2004 Writing Committee. *Circulation* 2008;**117**:296-329.

4. Wu E, Judd RM, Vargas JD, Klocke FJ, Bonow RO, Kim RJ. Visualisation of presence, location, and transmural extent of healed Q-wave and non-Q-wave myocardial infarction. *Lancet* 2001;**357**:21-28.

5. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS. 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.

6. Cuocolo A, Acampa W, Nicolai E, Pace L, Petretta M, Salvatore M. Quantitative thallium-201 and technetium 99m sestamibi tomography at rest in detection of myocardial viability in patients with chronic ischemic left ventricular dysfunction. *J Nucl Cardiol* 2000;**7**:8-15.

7. Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. *Stat Med* 2002;**21**:2917-2930.