

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Monitoring of Bone Marrow Cell Homing Into the Infarcted Human Myocardium

Michael Hofmann, Kai C. Wollert, Gerd P. Meyer, Alix Menke, Lubomir Arseniev, Bernd Hertenstein, Arnold Ganser, Wolfram H. Knapp and Helmut Drexler

Circulation 2005;111:2198-2202; originally published online Apr 25, 2005;

DOI: 10.1161/01.CIR.0000163546.27639.AA

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2005 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/cgi/content/full/111/17/2198>

Subscriptions: Information about subscribing to *Circulation* is online at
<http://circ.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, 351 West Camden Street, Baltimore, MD 21202-2436. Phone 410-5280-4050. Fax: 410-528-8550. Email:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/static/html/reprints.html>

Monitoring of Bone Marrow Cell Homing Into the Infarcted Human Myocardium

Michael Hofmann, MD, MS*; Kai C. Wollert, MD*; Gerd P. Meyer, MD; Alix Menke, MD; Lubomir Arseniev, MD; Bernd Hertenstein, MD; Arnold Ganser, MD; Wolfram H. Knapp, MD; Helmut Drexler, MD

Background—Intracoronary transfer of autologous bone marrow cells (BMCs) promotes recovery of left ventricular systolic function in patients with acute myocardial infarction. Although the mechanisms of this effect remain to be established, homing of BMCs into the infarcted myocardium is probably a critical early event.

Methods and Results—We determined BMC biodistribution after therapeutic application in patients with a first ST-segment–elevation myocardial infarction who had undergone stenting of the infarct-related artery. Unselected BMCs were radiolabeled with 100 MBq 2-[¹⁸F]-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) and infused into the infarct-related coronary artery (intracoronary; n=3 patients) or injected via an antecubital vein (intravenous; n=3 patients). In 3 additional patients, CD34-positive (CD34⁺) cells were immunomagnetically enriched from unselected BMCs, labeled with ¹⁸F-FDG, and infused intracoronarily. Cell transfer was performed 5 to 10 days after stenting. More than 99% of the infused total radioactivity was cell bound. Nucleated cell viability, comparable in all preparations, ranged from 92% to 96%. Fifty to 75 minutes after cell transfer, all patients underwent 3D PET imaging. After intracoronary transfer, 1.3% to 2.6% of ¹⁸F-FDG–labeled unselected BMCs were detected in the infarcted myocardium; the remaining activity was found primarily in liver and spleen. After intravenous transfer, only background activity was detected in the infarcted myocardium. After intracoronary transfer of ¹⁸F-FDG–labeled CD34–enriched cells, 14% to 39% of the total activity was detected in the infarcted myocardium. Unselected BMCs engrafted in the infarct center and border zone; homing of CD34–enriched cells was more pronounced in the border zone.

Conclusions—¹⁸F-FDG labeling and 3D PET imaging can be used to monitor myocardial homing and biodistribution of BMCs after therapeutic application in patients. (*Circulation*. 2005;111:2198-2202.)

Key Words: bone marrow cells ■ imaging ■ myocardial infarction ■ nuclear medicine

It has been proposed that cardiac transfer of bone marrow cells (BMCs) can be used for cardiac tissue repair and regeneration in patients after acute myocardial infarction (AMI).¹⁻³ This concept is supported by the recent randomized controlled BOne marrOw transfer to enhance ST-elevation infarct regeneration (BOOST) trial, showing that intracoronary transfer of unselected autologous BMCs during the early postinfarction period enhances recovery of left ventricular (LV) ejection fraction after 6 months.⁴ The mechanisms by which BMCs enhance functional recovery after AMI remain poorly understood. It has been proposed that bone marrow–derived endothelial progenitor cells may enhance neovascularization of ischemic tissues by differentiating to endothelial cells.⁵ In addition, recent articles have highlighted the potential of BMCs to secrete proangiogenic factors and suggested that paracrine signal-

ing,⁶ rather than transdifferentiation,^{7,8} may promote functional recovery after ischemic injury. In any case, BMC homing in the infarcted myocardium is probably a critical early event after intracoronary transfer. In animal models, myocardial homing of transplanted stem and progenitor cells has been monitored by fluorescence or radioactive labeling.⁹⁻¹¹ However, cell fate of BMCs after therapeutic application in patients has never been determined. We postulated that in vivo assessment of myocardial homing and biodistribution of transplanted BMCs may provide mechanistic insight into and help to optimize procedural aspects of BMC therapy in patients. In the present study, we used 2-[¹⁸F]-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) labeling and 3D PET imaging to monitor myocardial homing and biodistribution of autologous BMCs after therapeutic application in patients after AMI.

Received October 5, 2004; revision received January 10, 2005; accepted January 21, 2005.

From the Departments of Nuclear Medicine (M.H., W.H.K.), Cardiology and Angiology (K.C.W., G.P.M., A.M., H.D.), and Hematology and Oncology (L.A., B.H., A.G.), Hanover Medical School, Hanover, Germany.

*Drs Hofmann and Wollert contributed equally to this work.

The online-only Data Supplement, which contains 2 movies, can be found with this article at <http://www.circulationaha.org>.

Correspondence to Prof Dr Helmut Drexler, Abt Kardiologie und Angiologie, Medizinische Hochschule Hannover, Carl-Neuberg Str 1, 30625 Hannover, Germany. E-mail drexler.helmut@mh-hannover.de

© 2005 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000163546.27639.AA

Methods

Patient Population

Patients with a first ST-segment-elevation myocardial infarction who had undergone percutaneous coronary intervention with stent implantation of the infarct-related coronary artery and demonstrated hypokinesia or akinesia of more than two thirds of the LV antero-septal, lateral, and/or inferior wall (as revealed by angiography immediately after percutaneous coronary intervention) were included in the study. Patients presenting with Killip class III and IV symptoms or multivessel coronary artery disease were excluded. The same inclusion and exclusion criteria were used in the BOOST trial.⁴ All patients provided written informed consent and received optimal postinfarction pharmacotherapy, including aspirin, clopidogrel, ACE inhibitors or angiotensin receptor blockers, β -blockers, and statins. The study was approved by our local ethics committee (No. 3297).

BMC Preparation

Five to 10 days after percutaneous coronary intervention, bone marrow was aspirated from the posterior iliac crest under a brief general anesthesia with etomidate and midazolam. Unselected BMCs were enriched under good manufacturing practice conditions by 4% gelatin-polysuccinate density gradient sedimentation as described.⁴ CD34⁺ cells were immunomagnetically enriched from unselected BMCs by the CliniMACS^{plus} System and CD34 antibodies from Miltenyi Biotech. The number of CD34⁺ cells in unselected BMC preparations and in CD34-enriched preparations was determined by flow cytometry analysis (FACSCalibur, BD Biosciences) using an antibody from Beckman Coulter.

¹⁸F-FDG Radiolabeling

Unselected BMCs or CD34-enriched cells were incubated under sterile conditions in a 10-mL test tube with 100 MBq ¹⁸F-FDG (specific activity, 10 MBq/mL) for 30 minutes at 28°C under gentle rolling in serum-free PBS (pH 7.2) containing 10 U/mL heparin (Roche) and 0.1 U/mL recombinant human insulin (Novo Nordisk). To remove excess unbound ¹⁸F-FDG, cells were subjected to a 3-step centrifugation and washing process in heparinized PBS (7g, 27g, and 60g; 120 seconds each). Radioactivity in the supernatant and cell pellet was measured with a dose calibrator (Capintec). ¹⁸F-FDG-labeled cells were resuspended in 2 mL heparinized (10 U/mL) saline before application. Cell viability was assessed by trypan blue dye exclusion assay.

BMC Transfer

Protocol 1

Unselected BMCs were prepared from 126 to 145 mL bone marrow aspirate (n=3 patients). Five percent of the BMC preparation (vol/vol) was labeled with ¹⁸F-FDG. ¹⁸F-FDG-labeled cells were infused into the infarct-related artery via the central lumen of an over-the-wire balloon catheter (Concerto, Occam International) as described.⁴ To maximize the contact time of the BMCs with the microcirculation of the infarct-related artery, the balloon was inflated inside the stent for \approx 3 minutes during the infusion. Subsequently, nonlabeled BMCs (95% of the initial preparation) were infused during 3 to 4 additional coronary occlusions. Between occlusions, the coronary artery was reperfused for 3 minutes. Fifty-five to 75 minutes after intracoronary transfer, patients underwent 3D PET imaging (see below) to determine the biodistribution of ¹⁸F-FDG-labeled BMCs.

Protocol 2

Unselected BMCs were prepared from 120 to 131 mL bone marrow aspirate (n=3 patients). Five percent of the BMC preparation (vol/vol) was labeled with ¹⁸F-FDG. One half of the ¹⁸F-FDG-labeled cells were then injected via a right antecubital vein, followed by 3D PET imaging 50 to 60 minutes after injection. Subsequently, the second half of the ¹⁸F-FDG-labeled cells and the nonlabeled unselected BMCs (95% of the initial preparation) were infused into the

infarct-related artery as described above. Sixty to 70 minutes after the intracoronary transfer, patients underwent a second 3D PET scan.

Protocol 3

Unselected BMCs were prepared from 277 to 336 mL bone marrow aspirate (n=3 patients). Note that 2.5-fold more bone marrow was aspirated compared with protocols 1 and 2 to obtain sufficient numbers of CD34⁺ cells. CD34⁺ cells were enriched from unselected BMCs, labeled with ¹⁸F-FDG, and infused into the infarct-related artery. Afterward, 40% of the nonlabeled, CD34-depleted BMC fraction was infused during 3 to 4 additional coronary occlusions. Sixty to 75 minutes after intracoronary transfer, patients underwent 3D PET imaging.

3D PET Imaging

The 3D PET imaging (10-minute emission time, 5-minute transmission time) was performed with the patient in the supine position in a dedicated full-ring PET scanner (ECAT Exact 47, Siemens). After correction for half-life, attenuation, and scatter (as measured by a delayed coincidence channel), reconstruction was performed with the iterative algorithm OSEM 7.2 (CPS Innovations) with 6 iterations, 16 subsets, a 2.0-mm prereconstruction filter with a zoom of 1.5, and a 128 \times 128-pixel matrix. Activity concentrations were evaluated by the region-of-interest technique using manufacturer's software. In all patients, scans covering the upper abdomen and chest were obtained. Full 3D regions of interest were generated with the MEDx 3.0 software (Medical Numerics). Myocardial activity was normalized to the sum of activity in liver, spleen, and heart.

Results

Patient Characteristics

Nine patients (all male) were studied. Patient characteristics are summarized in Table 1. All patients tolerated BMC harvesting and transfer well. No bleeding complications at the harvest site were observed. There were no increases in troponin T serum levels in any of the patients 1 day after BMC transfer, indicating that the procedure did not inflict additional ischemic damage to the myocardium.

BMC Labeling With ¹⁸F-FDG

Labeling details are presented in Table 2. Nucleated cell viability, which was comparable in all preparations, ranged from 92% to 96%. In all applications, the activity in the supernatant after 3 steps of washing and centrifugation was $0.6\pm 0.3\%$ of the cell-bound activity.

Biodistribution of ¹⁸F-FDG-Labeled Unselected BMCs After Intracoronary Transfer (Protocol 1)

Radioactivity retention in the myocardium after intracoronary application of ¹⁸F-FDG-labeled unselected BMCs was 1.3% in patient 1, 2.6% in patient 2, and 2.3% in patient 3 (Table 2). In all 3 patients, activity in the heart was localized in only the area of the culprit vessel; no significant activity was detected in other regions of the myocardium. More than 85% of the activity in the field of view accumulated in liver and spleen (example shown in the Figure, A and B, and Movie I).

Biodistribution of Unselected ¹⁸F-FDG-Labeled BMCs After Intravenous Injection (Protocol 2)

Intravenous application of ¹⁸F-FDG-labeled BMCs resulted in activity accumulation predominantly in liver and spleen (>84% of the activity in the field of view). Myocardium and adjacent mediastinal tissues were not resolved visually because of a lack of contrast (Table 2). When ¹⁸F-FDG-labeled

TABLE 1. Demographic, Clinical, and Angiographic Characteristics of the Patients

Patient	Age, y	BMI, kg/m ²	Risk Factors	Time From Symptom Onset to PCI, h	Infarct-Related Artery	Maximum CK Level, U/L	TIMI Flow Grade Before/After PCI
1	61	28.2	C, H	3	LAD	6480	0/3
2	39	26.1	F,H,S	11	LCx	1767	2/3
3	59	29.1	C, H	22	LAD	464*	2/3
4	43	25.6	C, S	3	RCA	1530	2/3
5	39	32.8	C,D,H	8	LAD	1228	0/3
6	36	27.2	F, S	27	LAD	905*	0/3
7	49	28.1	C, H	5	LAD	4804	0/3
8	66	24.4	H	9	LAD	7479	2/2
9	41	28.7	S	3	LAD	5366	0/3

BMI indicates body mass index; PCI, percutaneous coronary intervention; C, total cholesterol level >200 mg/dL; H, arterial hypertension; LAD, left anterior descending artery; F, family history of premature coronary artery disease; S, current smoking; LCx, left circumflex artery; D, diabetes mellitus; and RCA, right coronary artery.

*Because of late presentation of these 2 patients, CK levels measured on admission are shown.

BMCs were subsequently infused intracoronarily in the same 3 patients, a significant retention in radioactivity was observed in the myocardium: 1.8% in patient 4, 4.3% in patient 5, and 5.3% in patient 6.

Biodistribution of ¹⁸F-FDG-Labeled CD34-Enriched BMCs After Intracoronary Transfer (Protocol 3)

Radioactivity retention in the myocardium after intracoronary application of ¹⁸F-FDG-labeled CD34-enriched BMCs was 39% in patient 7, 14% in patient 8, and 24% in patient 9 (Table 2). In all 3 patients, activity in the heart was localized

in the area of the culprit vessel only. No significant activity was detected in other regions of the myocardium. More than 55% of the activity in the field of view accumulated in liver and spleen (example shown in the Figure, C and D, and Movie II). In contrast to unselected BMCs, which homed to the infarct center and border zone (Figure, A and B), homing of CD34-enriched BMCs was most prominent in the infarct border zone (Figure, C and D, and Movie II).

Discussion

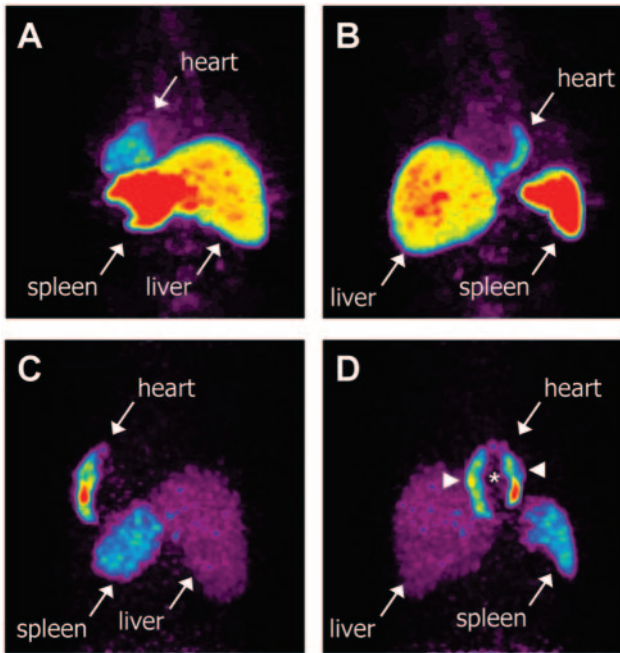
Radiolabeling of leukocytes is an established nuclear medicine procedure to localize areas of infection and inflammation

TABLE 2. BMC Preparation and ¹⁸F-FDG Labeling

Patient	Initial BM Aspirate, mL	Final BMC Preparation, After Gelatine Sedimentation in Protocols 1 and 2 and After Gelatine Sedimentation and CD34 Selection in Protocol 3					Labeling and Cell Transfer		
		Cell Fraction	Volume, mL	NCs, ×10 ⁸	CD34 ⁺ , ×10 ⁶	CD34 ⁺ , %	Labeled Cell Population	Specific Activity, Bq/10 ³ NCs	Myocardial Homing,* % of cells
Protocol 1: labeling of unselected BMCs followed by intracoronary transfer									
1	143	Unselected	29	36.7	14.9	0.55	5% of unselected BMCs	0.4	1.3
2	145	Unselected	26	21.1	13.5	0.57	5% of unselected BMCs	1.8	2.6
3	126	Unselected	25	18.5	8.7	0.62	5% of unselected BMCs	0.8	2.3
Protocol 2: labeling of unselected BMCs followed by intravenous (first half) and intracoronary (second half) transfer									
4	121	Unselected	25	20.9	10.4	0.50	5% of unselected BMCs	1.3	0 (after intravenous), 1.8 (after intracoronary)
5	120	Unselected	28	25.5	4.0	0.14	5% of unselected BMCs	0.6	0 (after intravenous), 4.3 (after intracoronary)
6	131	Unselected	21	26.5	8.6	0.61	5% of unselected BMCs	1.0	0 (after intravenous), 5.3 (after intracoronary)
Protocol 3: labeling of CD34-selected cells followed by intracoronary transfer									
7	336	CD34 ⁺	3	0.30	21.3	70.3	100% of CD34 ⁺ fraction	10	39
		CD34 ⁻	90	45.5	6.4	0.14			
8	320	CD34 ⁺	3	0.20	14.7	74.4	100% of CD34 ⁺ fraction	18	14
		CD34 ⁻	110	42.5	1.3	0.03			
9	277	CD34 ⁺	3	0.22	12.0	55.0	100% of CD34 ⁺ fraction	21	24
		CD34 ⁻	60	41.8	5.2	0.13			

BM denotes bone marrow; NCs, nucleated cells.

**P*=0.0036 for interprotocol comparisons (nonparametric exact Kruskal-Wallis test).



Myocardial homing and biodistribution of ^{18}F -FDG-labeled BMCs. Left posterior oblique (A) and left anterior oblique (B) views of chest and upper abdomen of patient 2 taken 65 minutes after transfer of ^{18}F -FDG-labeled, unselected BMCs into left circumflex coronary artery. BMC homing is detectable in the lateral wall of the heart (infarct center and border zone), liver, and spleen. Left posterior oblique (C) and left anterior oblique (D) views of chest and upper abdomen of patient 7 taken 70 minutes after transfer of ^{18}F -FDG-labeled, CD34-enriched BMCs into left anterior descending coronary artery. Homing of CD34-enriched cells is detectable in the anterosseptal wall of the heart, liver, and spleen. CD34⁺ cell homing is most prominent in infarct border zone (arrowheads) but not infarct center (asterisk).

in patients. Of the different labeling strategies for identification of inflammatory foci, ^{18}F -FDG PET provides superior image quality with high spatial resolution and adequate scatter and attenuation correction.¹² As shown in the present study, ^{18}F -FDG labeling followed by 3D PET imaging can be used to monitor myocardial homing and biodistribution of BMCs after therapeutic application in post-AMI patients.

Intracoronary delivery of unselected BMCs has recently been shown to enhance LV ejection fraction recovery in patients after AMI.⁴ Using the same methodology for isolation and intracoronary transfer of unselected BMCs that was used in the BOOST trial,⁴ we have shown that only a small fraction (1.3% to 2.6%) of the transplanted cells are actually retained in the infarcted myocardium, whereas most cells home to the liver and spleen within ≈ 1 hour after intracoronary delivery. Selective, stop-flow balloon catheter delivery of BMCs resulted in BMC homing only in myocardial regions perfused by the infarct-related artery. Assuming that it is the engrafted cell population that promotes functional recovery after AMI, we postulated that ^{18}F -FDG labeling can be used to explore alternative, less invasive routes of BMC application after AMI. However, after intravenous application of ^{18}F -FDG-labeled BMCs, no activity retention was detected in the myocardium. This finding may not be too surprising when we consider that no coronary stop-flow technique was used and that only $\approx 4\%$ to 5% of cardiac output

passes through the coronary arteries (even less through the culprit vessel). Similar to the situation after intracoronary BMC transfer, liver and spleen were the major sites of BMC homing after intravenous application, which is reminiscent of leukocyte scanning in which liver and spleen very effectively extract white blood cells during first passage.¹³ These data indicate that intracoronary transfer may be the preferred route for therapeutic BMC delivery in patients after AMI, although the limited time interval between administration and imaging did not allow us to exclude late redistribution of cells to the infarcted myocardium.

Unselected BMCs represent a mixed population of various stem and progenitor cells, stromal cells, and hematopoietic cells at various maturation stages. It is not yet clear which cell population(s) promote functional recovery in patients after intracoronary transfer. In experimental models of AMI, functional improvements have been reported after transplantation of unselected or highly selected BMC populations, including endothelial progenitor cells, hematopoietic stem cells, and mesenchymal stem cells.^{9,14–17} Importantly, by delivering proangiogenic factors, subsets of mature hematopoietic cells may cooperate with transplanted or resident cardiac stem and progenitor cells to enhance their capacity for tissue repair after ischemic injury.^{18–21}

After myocardial ischemia and reperfusion, neutrophils and monocytes are recruited to the infarcted myocardium as part of a cell-mediated inflammatory response,²² suggesting that myocardial homing of ^{18}F -FDG-labeled unselected BMCs may reflect, to some degree, homing of such differentiated cell types. However, as our study shows, cells expressing the stem and progenitor cell surface marker CD34 also engraft in the infarcted human myocardium. In fact, CD34-enriched cells displayed a higher retention in the infarcted myocardium compared with unselected BMCs. However, this may be due in part to the fact that, on average, 5-fold-more ^{18}F -FDG-labeled unselected compared with CD34-enriched BMCs were infused intracoronarily, which may have led to a saturation of binding sites by unselected BMCs. Interestingly, CD34-enriched cells predominantly homed in the infarct border zone. Notably, intracoronary BMC transfer enhances LV contractility primarily in myocardial segments adjacent to the infarcted area,⁴ consistent with the idea that homing of CD34⁺ BMCs corresponds to late functional improvement.

Compared with other PET tracers, ^{18}F -FDG has a number of important advantages, including availability and ease of use, very low concentrations needed for PET imaging (femtomolar range), and long positron range of the emitted β -particle (≈ 0.5 mm), which results in a radiation dose deposition mostly outside the labeled cells. Moreover, the 511-keV γ -photons emitted after positron annihilation display very low absorption in water and contribute $<5\%$ to the dose absorbed. Because the exposure of cells to radioactivity is highest during the labeling step and very low after transfer into the patient, the major dosage is absorbed during labeling and purification. Using the MIRD 3.1 software package,²³ we estimated that the dose distributed in the labeling volume is <0.05 Gy. At a hematocrit of 10% (for BMCs), the homogeneously suspended cells absorb only a fraction of the energy deposited, leading to radiation doses of <0.005 Gy. This dose is well within the range of other nuclear medicine applications such as ^{18}F -FDG PET or ^{111}In -octreotide

scintigraphy.^{24,25} This is in contrast to the frequently used auger-electron emitter tracer ¹¹¹In-oxine, which, because of the short range of the emitted low-energy β^- -particles, delivers most of its radiation dose inside the labeled cells. This may explain why higher cellular toxicity has been observed with this tracer.¹¹

Because of the physical half-life (110 minutes) of ¹⁸F, we could not assess cell trafficking and myocardial retention of BMCs days after therapeutic application. Other PET isotopes with longer half-lives like ¹²⁴I, ⁸⁶Y, ⁶⁶Ga, or ⁶⁴Cu have unfavorable branching ratios or additional image-disturbing emissions but may be worth pursuing because of the small doses needed to monitor cell distribution. Another way to gain insight into more delayed time frames is to use PET scanners with higher performances at very low intracorporal activity concentrations that display improved intrinsic signal-to-noise ratios (eg, LSO or GSO detectors). With such techniques, time points up to 10 hours after cell application (5 times the physical half-life of ¹⁸F) would be accessible for quantification.

Whether the amount of BMCs or CD34⁺ cells retained in the infarcted myocardium is correlated to later improvement in LV contractile function will have to be addressed in future studies. If that were the case, ¹⁸F-FDG PET scanning could be used to screen various pharmacological or genetic approaches that may enhance myocardial homing and therapeutic efficacy of transplanted bone marrow–derived cell populations.

Acknowledgment

This work was supported in part by a grant from the Novartis Foundation.

Disclosure

Dr Arseniev is business leader of Cytonet Hannover, the company that prepared the bone marrow cells. Dr Arseniev has not been involved in data analyses.

References

1. Strauer BE, Brehm M, Zeus T, Kosterling M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation*. 2002;106:1913–1918.
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. Wollert KC, Drexler H. Clinical applications of stem cells for the heart. *Circ Res*. 2005;96:151–163.
4. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised-controlled clinical trial. *Lancet*. 2004;364:141–148.
5. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res*. 2004;95:343–353.
6. Heil M, Ziegelhoeffer T, Mees B, Schaper W. A different outlook on the role of bone marrow stem cells in vascular growth: bone marrow delivers software not hardware. *Circ Res*. 2004;94:573–574.
7. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JJ, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004;428:664–668.
8. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman JL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*. 2004;428:668–673.
9. Koehler AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow–derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med*. 2001;7:430–436.
10. Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, Ecker T, Henze E, Zeiher AM, Dimmeler S. Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation*. 2003;107:2134–2139.
11. Brenner W, Aicher A, Ecker T, Massoudi S, Zuhayra M, Koehl U, Heeschen C, Kampen WU, Zeiher AM, Dimmeler S, Henze E. ¹¹¹In-labeled CD34⁺ hematopoietic progenitor cells in a rat myocardial infarction model. *J Nucl Med*. 2004;45:512–518.
12. Becker W, Meller J. The role of nuclear medicine in infection and inflammation. *Lancet Infect Dis*. 2001;1:326–333.
13. Datz FL, Luers P, Baker WJ, Christian PE. Improved detection of upper abdominal abscesses by combination of ^{99m}Tc sulfur colloid and ¹¹¹In leukocyte scanning. *Am J Roentgenol*. 1985;144:319–323.
14. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 2001;104:1046–1052.
15. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002;105:93–98.
16. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, Uchida S, Masuo O, Iwaguro H, Ma H, Hanley A, Silver M, Kearney M, Losordo DW, Isner JM, Asahara T. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*. 2003;107:461–468.
17. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med*. 2003;9:1195–1201.
18. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci U S A*. 1997;94:663–668.
19. Tordjman R, Delaire S, Plouet J, Ting S, Gaulard P, Fichelson S, Romeo PH, Lemarchand V. Erythroblasts are a source of angiogenic factors. *Blood*. 2001;97:1968–1974.
20. Rehman J, Li J, Orschell CM, March KL. Peripheral blood “endothelial progenitor cells” are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation*. 2003;107:1164–1169.
21. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med*. 2003;9:702–712.
22. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res*. 2002;53:31–47.
23. Stabin MG. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med*. 1996;37:538–546.
24. Deloar HM, Fujiwara T, Shidahara M, Nakamura T, Watabe H, Narita Y, Itoh M, Miyake M, Watanuki S. Estimation of absorbed dose for 2-[¹⁸F]-fluoro-2-deoxy-D-glucose using whole-body positron emission tomography and magnetic resonance imaging. *Eur J Nucl Med*. 1998;25:565–574.
25. Krenning EP, Bakker WH, Kooij PP, Breeman WA, Oei HY, de Jong M, Reubi JC, Visser TJ, Bruns C, Kwakkeboom DJ, Reijs AE, van Hagen PM, Koper JW, Lamberts SW. Somatostatin receptor scintigraphy with indium-111-DTPA-D-Phe-1-octreotide in man: metabolism, dosimetry and comparison with iodine-123-Tyr-3-octreotide. *J Nucl Med*. 1992;33:652–658.