Infarct Size Determines Myocardial Uptake of CD34+ Cells in the Peri-Infarct Zone

Results From a Study of 99mTc-extametazime–Labeled Cell Visualization Integrated With Cardiac Magnetic Resonance Infarct Imaging

Piotr Musialek, MD, DPhil; Lukasz Tekieli, MD; Magdalena Kostkiewicz, MD, PhD; Tomasz Miszalski-Jamka, MD; Piotr Klimeczek, MD; Wojciech Mazur, MD; Wojciech Szot, MD; Marcin Majka, DSc, PhD; R. Pawel Banys, MSc; Danuta Jarocha, PhD; Zbigniew Walter, MD; Maciej Krupinski, MD; Piotr Pieniazek, MD, PhD; Maria Olszowska, MD, PhD; Krzysztof Zmudka, MD, PhD; Mieczyslaw Pasowicz, MD, PhD; Dean J. Kereiakes, MD; Wieslawa Tracz, MD, PhD; Piotr Podolec, MD, PhD; Wojciech Wojakowski, MD, PhD

Background—Effective progenitor cell recruitment to the ischemic injury zone is a prerequisite for any potential therapeutic effect. Cell uptake determinants in humans with recent myocardial infarction are not defined. We tested the hypothesis that myocardial uptake of autologous CD34+ cells delivered via an intracoronary route after recent myocardial infarction is related to left ventricular (LV) ejection fraction (LVEF) and infarct size.

Methods and Results—Thirty-one subjects (age, 36–69 years; 28 men) with primary percutaneous coronary intervention–treated anterior ST-segment–elevation myocardial infarction and significant myocardial injury (median peak troponin I, 138 ng/mL [limits, 58–356 ng/mL]) and sustained LVEF depression at ≤54% were recruited. On day 10 (days 7–12), 4.3×10⁶ (0.7–9.9×10⁶) 99mTc-extametazime–labeled autologous bone marrow CD34+ cells (activity, 77 MBq [45.9–86.7 MBq]) were administered transcoronarily (left anterior descending coronary artery). 99mTc-methoxyisobutyl isonitrile (99mTc-MIBI) single-photon emission computed tomography before cell delivery showed 7 (2–11) (of 17) segments with definitely abnormal/absent perfusion. Late gadolinium-enhanced infarct core mass was 21.7 g (4.4–45.9 g), and infarct border zone mass was 29.8 g (3.9–60.2 g) (full-width at half-maximum, signal intensity thresholding algorithm). One hour after administration, 5.2% (1.7%–9.9%) of labeled cell activity localized in the myocardium (whole-body planar γ scan). Image fusion of labeled cell single-photon emission computed tomography with LV perfusion single-photon emission computed tomography or with cardiac magnetic resonance infarct imaging indicated cell uptake in the peri-infarct zone. Myocardial uptake of labeled cells activity correlated in particular with late gadolinium-enhanced infarct border zone mass (r=0.84, P<0.0001); it also correlated with peak TnI (r=0.76, P<0.001), severely-abnormal/absent perfusion segment number (r=0.45, P=0.008), and late gadolinium-enhanced infarct core (r=0.58, P=0.0003) but not with echocardiography LVEF (r=0.07, P=0.68) or gated single-photon emission computed tomography LVEF (r=0.28, P=0.16). The correlation with cardiac magnetic resonance imaging–LVEF was weak (r=–0.38; P=0.04).

Conclusions—This largest human study with labeled bone marrow CD34+ cell transcoronary transplantation after recent ST-segment–elevation myocardial infarction found that myocardial cell uptake is determined by infarct size rather than LVEF and occurs preferentially in the peri-infarct zone. (Circ Cardiovasc Imaging. 2013;6:320-328.)

Key Words: cardiac magnetic resonance imaging ■ CD34+ cells ■ gadolinium late-enhancement ■ infarct border zone ■ myocardial infarction–cellular therapy ■ progenitor cell tracking ■ single-photon emission computed tomography ■ transcoronary cell transplantation

March 2013

DOI: 10.1161/CIRCIMAGING.112.979633

Circ Cardiovasc Imaging is available at http://circimaging.ahajournals.org

Circ Cardiovasc Imaging is available at http://circimaging.ahajournals.org

© 2012 American Heart Association, Inc.
Propenitor cell therapy is emerging as a promising therapeutic modality in patients with acute myocardial infarction (MI) and substantial myocardial injury, and over the last 10 years, >1500 patients were evaluated in controlled, clinical trials that included transcoronary cell delivery. So far, the key primary endpoint in cell therapy trials has been an improvement in left ventricular (LV) ejection fraction (LVEF). REPAIR-AMI, a placebo-controlled clinical study with transcoronary administration of bone marrow (BM)-derived mononuclear cells indicated a modest (2.5%) absolute increase in LVEF at 4 months compared with placebo; however, several other studies showed no significant benefit of such treatment.

Recent animal studies have indicated that the mechanism by which BM cells exert their beneficial effect may be largely dependent on the paracrine actions of engrafted cells. In particular, BM cells secrete several substances that inhibit apoptosis, activate resident cardiac stem cells, and stimulate angiogenesis. Because effective recruitment of progenitor cells to the injury zone is a prerequisite for any potential therapeutic effect, it is essential to define the localization and determinants of myocardial cell uptake in humans. Pilot studies suggested that selected BM CD34+ cells might exhibit higher engraftment capacity than nonselected BM mononuclear cells. Nevertheless, animal and human studies with labeled progenitor cells have thus far included 1 to a maximum of 8 subjects with recent MI precluding any meaningful systematic analysis of the potential relationship between the extent of myocardial injury and cell uptake. On the contrary, pivotal clinical studies have used LVEF (typically ≤45%) as the main inclusion criterion and efficacy endpoint in the degree of LVEF impairment. Therefore, the role of LVEF as a main inclusion criterion and study outcome measure in progenitor cell myocardial therapy trials requires validation.

We used integrated imaging, involving cardiac magnetic resonance (cMRI) as the reference technique for MI and contractility evaluation and single-photon emission computed tomography (SPECT) for high-sensitivity visualization of labeled cells to evaluate the engraftment of autologous CD34+ BM cells after transcoronary delivery in a series of consecutive patients with recent ST-segment–elevation MI (STEMI) and substantial impairment of LV contractility maintained ≈10 days after STEMI.

Methods

Study Population
Consecutive patients with first STEMI (anterior location; left anterior descending coronary artery as the infarct-related artery) treated with primary percutaneous coronary intervention (pPCI) and LVEF ≤45% were screened for the study (see study flow chart in Figure 1). Troponin I (TnI) was evaluated on admission, every 8 hours for the first 72 hours, and then every 24 hours until discharge to determine the peak TnI level. Echocardiography was repeated before study enrollment to exclude subjects with spontaneous recovery of LV contractility. Only patients meeting the inclusion criteria—peak TnI level ≥50 ng/mL (consistent with substantial myocardial injury) and sustained reduction of LVEF ≤45% on the study enrollment day (5–10)—were recruited. Of the 50 consecutive patients with LVEF ≤45% at 24 hours after pPCI, 16 (32%) did not meet the criteria of significant TnI release and persistent depression of LV systolic function (LVEF ≤45%; see Videos I–IV in the online-only Data Supplement indicating how inclusion of subjects with acutely depressed LVEF in absence of significant injury manifest by myonecrotic marker release might confound study data). Three patients could not undergo MRI (metal implant in 1, claustrophobia in 2). Thus, the study population included 31 patients (28 men; age, 36–69 years; median, 58 years).

Cell Harvesting, Isolation, Labeling, and Transfer
Cell transfer was performed 7 to 12 days after pPCI. On the morning of the day of cell administration, BM (80–120 mL) was harvested from the iliac crest. Mononuclear cells were separated with Ficoll, consistent with the REPAIR-AMI and REGENT protocols. The CD34+ cells were selected immunomagnetically with monoclonal antibodies coupled with magnetic beads (MACS, Miltenyi Biotec GmbH), and the number of CD34+CD45RO− cells was determined by fluorescence-activated cell sorter analysis.2 The median number of mononuclear cells was 3.5×10^6 (range, 0.85×10^6–10×10^6); the median CD34+ cell yield was 4.3×10^6 (range, 0.70×10^6–9.90×10^6); and a median of 2.1×10^6 cells (range, 0.30×10^6–6.79×10^6) were CD34+/CXCR4 positive. The CD34+ cell purity by fluorescence-activated cell sorter analysis was 91% to 96%. For transcoronary transfer, the CD34+ pool of cells was used. The cells were labeled with 99mTc-exametazime (Ceretec, Amersham), similar to previously described protocols for labeling endothelial progenitor cells with 99mTc-exametazime. In brief, cells were incubated for 30 minutes with 99mTc-exametazime (110 MBq); washed 3 times and resuspended in 30 mL heparinized (10 IU/mL) saline containing human albumin, and were administered via the infarct-related artery (left anterior descending coronary artery) with a perfusion catheter (30-mL cell suspension at 3 bar). Radioactivity of the labeled cells was determined with a rate meter. Before their transcoronary administration, the viability of labeled cells was assessed (trypan blue dye exclusion assay). To identify any significant effect of the labeling procedure on the migratory capacity of the cells in a subset of subjects (n=6), we used a Matrigel...
cell migration assay to evaluate labeled cell migration to stromal cell–derived factor 1 at 1 to 4 hours after labeling.

**Myocardial Imaging Studies**

Myocardial perfusion was evaluated by SPECT (⁹⁹Tc-MIBI; dual-head camera E.CAM; Siemens; 64 projections; 25 seconds per stop; 32 stops over 180°; matrix size, 64x64; photpeaks set at 140 keV) to 36 to 48 hours before cell delivery. Images were analyzed with the Autoperfusion Protocol (Siemens). The magnitude of perfusion defect was determined in a 17-segment model according to the American Heart Association/American College of Cardiology/American Society of Nuclear Cardiology 5-point scoring system (0=normal perfusion; 1=mild reduction in counts/minimally abnormal; 2=moderate reduction in counts/definitely abnormal; 3=severe reduction in counts; 4=absent uptake). Each segment was evaluated (ICON workstation, Siemens) by a consensus of 2 observers blinded to patient data, and the number of segments with definitely abnormal to absent perfusion (perfusion deficit score ≥2) was taken as an index of infarct injury by SPECT. In addition, gated SPECT (G-SPECT) was used to determine LV end-diastolic volume, LV end-systolic volume, and LVEF.

Late gadolinium-enhanced (LGE) images were obtained 10 to 15 minutes after a peripheral bolus injection of 0.2 mmol/kg Gd-DTPA (Magnevist; Schering; Germany; Turbo-FLASH sequence; slice thickness, 8 mm; gap, 0 mm; in-plane resolution, 1.4x1.4 mm). LGE images were analyzed with Medis Qmass MR version 7.5 software according to a previously validated algorithm. In brief, the infarct core (LGE_CORE) was defined as myocardium with signal intensity (SI) ≥50% of the maximal myocardial SI (full-width at half-maximum method) adjusted to include, if present, microvascular obstruction zones. Infarct border zone (gray zone; LGE_IBZ) was defined as the myocardium with SI greater than the peak SI in remote normal myocardium but <50% of maximal SI of the high SI myocardium outward to the LGE zone. The MRI measurements were taken by a consensus of 2 independent, experienced cMRI analysts blinded to the SPECT and clinical data.

**Quantification of Early Myocardial Retention of CD34+ Cells**

Whole-body planar (anterior) images were obtained with 1 head of the dual-head γ camera (E.CAM; Siemens; scanning speed, 12 cm/min), and the image acquisition was 1 hour after transcoronary cell transplantation. Quantification of early myocardial uptake of the labeled CD34+ cell activity was performed offline according to established methods using contour detection for each region of interest (eg, Figure 2A). The number of counts (mean value for the region of interest contour delineation performed 3 times by a consensus of 2 observers blinded to clinical data) was expressed as percent of total counts on whole-body images. Consistent with previous protocols, the myocardial activity uptake was taken as an indicator of
percent early myocardial retention of the labeled cells. By indexing to the individual total body activity, the between-subject assessment of the magnitude of cell uptake was independent of the differences in the injected activity. Label uptake in other organs was also determined. Because previous work showed that 1 hour after injection of \(^{99m}\text{Tc}\)-extametazime–labeled cells the total circulating blood (≈5 L) activity is at the level of ≈15% injected activity, the activity from the blood present in the heart was considered negligible.\(^{11,13}\)

Visualization of Myocardial Uptake of CD34\(^+\) Cells

After the whole-body scan, SPECT images of labeled cells activity were obtained for each subject using the same acquisition protocol as for perfusion imaging. The zone(s) of myocardial uptake of CD34\(^+\) cells were demonstrated qualitatively offline by integrating SPECT images of labeled cells activity with SPECT images of myocardial perfusion (eg, in Figure 2B) and with MRI LGE images (eg, in Figure 3) using a semiautomated algorithm aligning the 2 data sets with matching seed points including the apex and mitral valve annulus (3D-CardioFusion Software, Leonardo Workstation VE30A/VE30B, SYNGO MMWP, Siemens).

The study protocol conforms to the Declaration of Helsinki and was approved by the Jagiellonian University Ethical Committee. All subjects gave informed written consent.

Statistical Analysis

Data were evaluated with Statistica 9.0 (StatSoft). Continuous variables were expressed as median (limits); categorical data were shown as the number (percentage). Correlations were analyzed by linear regression with the Spearman \((r_s)\) or Pearson \((r_p)\) coefficient as appropriate and were shown as a plot. The tests were 2 tailed, and the significance level was defined as \(P<0.05\).

Results

Clinical and laboratory characteristics of the study group are shown in the Table. Median peak TnI level was 137 ng/mL (58.3–356 ng/mL). Median values of qualifying LVEF were 37% (23%–44%) by echocardiography, 34% (17%–48%) by G-SPECT, and 38% (21%–48%) by cMRI.

Cell Viability and Migratory Capacity

There was a small but significant reduction in cell viability associated with the labeling procedure (median, 98% before labeling versus 96% after labeling; \(P<0.05\)). Cell labeling protocol with 30 minutes of incubation with \(^{99m}\text{Tc}\)-extametazime (110 MBq) was used because we found that prolonging cell incubation with \(^{99m}\text{Tc}\)-extametazime to 60 minutes did not lead to a substantial increase in the labeling efficiency (median increase, 2.3%; range, 0.4%–4.2%) but was associated with a reduction in cell viability (up to 3.0%; median reduction, 1.8%; \(n=6\)). Spontaneous efflux (leak) of \(^{99m}\text{Tc}\) from the labeled cells was 6.2% (3.4%–9.1%; \(n=6\)) at 90 minutes. Migratory capacity of \(^{99m}\text{Tc}\)-extametazime–labeled CD34\(^+\) cells to stromal cell–derived factor 1 was similar to that of nonlabeled cells (26%–37% versus 29%–34% migrating cells, Matrigel cell migration assay).\(^{22,23}\)

CD34\(^+\) Cells Label Activity Uptake

The median activity of labeled cells was 76.9 MBq (45.9–86.7 MBq). The efficiency of \(^{99m}\text{Tc}\)-extametazime cell labeling was 69% (43%–78%), consistent with previous animal study reports.\(^8\)

Figure 2A shows typical whole-body (planar) \(\gamma\) scan 1 hour after transcoronary delivery of \(^{99m}\text{Tc}\)-extametazime–labeled autologous CD34\(^+\) cells. Examples of raw SPECT data of myocardial perfusion (\(^{99m}\text{Tc}\)-MIBI, 36 hours before cell delivery) and \(^{99m}\text{Tc}\)-extametazime–labeled CD34\(^+\) cells myocardial activity 1 hour after cell delivery (in the same subject) are displayed in Figure 2B, which shows cell uptake in the peri-infarct zone of reduced (but present) perfusion. Figure 3 shows typical gadolinium-enhanced cMRI images (top) and fused images of gadolinium-enhanced cMRI and autologous \(^{99m}\text{Tc}\)-extametazime–labeled CD34\(^+\) cell activity (bottom). On fused images of SPECT (cells) and cMRI (LGE), the cells appeared as clouds in the border area of the LGE zones (Figure 3). The 4-fold difference in resolution of SPECT compared with MRI\(^{24}\) (7.4 versus 1.8 mm) and the fact that 3-dimensional SPECT (in contrast to planar scintigraphy) is not quantitative for cell label imaging precluded any further detailed analysis of SPECT–demonstrated cell uptake areas in relation to the infarct core zone versus border zone by cMRI\(^{19,20}\).}

The number of labeled CD34\(^+\) cells correlated with their total activity \((r=0.72, P=0.0007)\). However, there was no relationship between the activity per million cells and percent myocardial activity uptake \((r=-0.038, P=0.834)\), allowing...
### Table. Demographic, Clinical, and Laboratory Data for the Study Group, and the CD34+ Cells Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58 (36–69)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>14 (45.2)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>7 (22.6)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>26 (83.9)</td>
</tr>
<tr>
<td>Smoking (h/o or current), n (%)</td>
<td>2 (6.4)</td>
</tr>
<tr>
<td>BMI &gt;30 kg/m², n (%)</td>
<td>2 (6.4)</td>
</tr>
<tr>
<td>eGFR &lt;90 mL/kg/min, n (%)</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>Time from the onset of symptoms to pPCI, h</td>
<td>6 (3–13)</td>
</tr>
<tr>
<td>Glycoprotein IIb/IIa inhibitor before/at pPCI</td>
<td>27 (87.1)</td>
</tr>
<tr>
<td>Infarct-related artery=proximal LAD, n (%)</td>
<td>31 (100)</td>
</tr>
<tr>
<td>STEMI to cell transfer, d</td>
<td>10 (7–12)</td>
</tr>
<tr>
<td>Peak troponin I, ng/mL</td>
<td>137 (58.3–356)</td>
</tr>
<tr>
<td>LVEF,* %, by echocardiography</td>
<td>37 (23–44)</td>
</tr>
<tr>
<td>LVEF,* %, by G-SPECT†</td>
<td>34† (17–48)</td>
</tr>
<tr>
<td>Infarct-related artery=proximal LAD, n (%)</td>
<td>31 (100)</td>
</tr>
<tr>
<td>Labeled CD34+ cell viability, %</td>
<td>96 (91–99)‡</td>
</tr>
<tr>
<td>Labeled CD34+ cell activity, MBq</td>
<td>77 (45.9–86.7)</td>
</tr>
<tr>
<td>Early myocardial uptake of activity, %*</td>
<td>5.2 (1.7–9.9)</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; cMRI, cardiac magnetic resonance imaging; eGFR, estimated glomerular filtration rate; G-SPECT, gated single-photon emission computed tomography; h/o, history of; LAD, left anterior descending coronary artery; LVEF, left ventricular ejection fraction; and pPCI, primary percutaneous coronary intervention. Values are median (limit) when appropriate.

*Qualifying values at 24 to 48 hours before cell delivery.
†n=4 nongated because of arrhythmia.
‡P<0.05 versus viability before labeling.
#At 60 minutes after transcoronary cell delivery; fraction of total body activity on whole-body γ scan (see text for details).

between-subject comparisons of the proportion of cell label uptake by the myocardium.13

One hour after transcoronary cell delivery, myocardial activity uptake was 1.7% to 9.9% (Table). Median activity uptake in other organs was as follows: liver, 25.6%; lungs, 16.0%; spleen, 11.4%; bowel, 1.7%; and urinary bladder, 2.1%.

### Determinants of Myocardial Cell Uptake

#### Left Ventricular Ejection Fraction

Myocardial uptake of 99mTc-labeled cells activity showed no significant correlation with LVEF measured either by echocardiography (r=−0.07, P=0.68) or by G-SPECT LVEF (r=−0.28, P=0.16); however, a weak correlation with the extent of global LV systolic dysfunction measured by cMRI (r=−0.38, P=0.04) was observed.

#### Infarct Core and Infarct Border Zone Size

Median infarct core mass by LGE cMRI was 21.7 g (4.4–45.9 g) and the infarct border zone mass was 29.8 g (3.9–60.2 g); this corresponded to a median of 11.6% and 15.7% of the total LV mass, respectively. On SPECT, the number of LV segments with definitely abnormal to absent perfusion (perfusion deficit score ≥2) was 7 (2–11) of the total 17, and there was a significant correlation between LGEcore and the proportion of LV with perfusion deficit score ≥2 (r=0.57, P<0.001).

In contrast to LVEF, there was a significant positive correlation between infarct size by peak TnI and the myocardial activity of 99mTc-labeled cells (r=0.76, P=0.0001; Figure 4A). Moreover, the magnitude of early myocardial retention of 99mTc-labeled cells activity was also related to the number of LV segments with perfusion deficit score ≥2 (r=0.45; P=0.0075; Figure 4B). The labeled cell activity uptake correlated with LGEcore (r=0.58; P=0.0003; Figure 4C) and, in particular, with LGEIBZ (r=0.84; P<0.0001; Figure 4D). This correlation was maintained when the size of infarct core and infarct border zone were expressed as percent LV mass (r=0.57 and r=0.62, respectively; P<0.001 for both). On the day of index cMRI (day 6–11 of STEMI; Figure 1), microvascular obstruction by cMRI was present in 18 patients (58%). The median microvascular obstruction mass was 6.8 g (0.63–28.8 g), and the size of microvascular obstruction was not related to the magnitude of myocardial uptake of labeled cells (r=0.14, P=0.58).

### Discussion

The main novel finding from this study is that the magnitude of myocardial uptake of intracoronary-delivered 99mTc-labeled autologous CD34+ cells is strongly correlated with several measures of infarct size, including in particular the infarct border zone size by cMRI ≤24 hours before the cell delivery, which occurred 7 to 12 days after pPCI. This correlation was also evident when infarct size was expressed as peak TnI concentration or the number of LV segments with severely impaired or absent perfusion on SPECT. Moreover, fused SPECT (cells)/SPECT (myocardial perfusion) or SPECT (cells)/cMRI (infect) images qualitatively indicated early cell retention in the peri-infarct zone (Figures 2B and 3), the size of which showed a particularly strong correlation with cell uptake (Figure 4D). In contrast, we were unable to identify a relationship between the uptake of transcoronary-delivered cells and LVEF determined by echocardiography or G-SPECT, and myocardial cell activity uptake was only weakly related to LVEF by the current gold standard, cMRI. Although the present work has not tested whether the uptake of BM–harvested CD34+ cells by the infarct-injured myocardium is different from that of circulating inflammatory cells,25 the present findings are consistent with a biological relevance of the infarct size–dependent CD34+ early uptake in the peri-infarct zone in humans.

### Mechanisms Underlying the Relationship Between Infarct Size and BM CD34+ Cell Uptake

Progenitor cell homing to the injury zone involves cell adhesion to the activated endothelium, extravasation, and migration along the gradient of chemottractants released by the myocardium in response to ischemia.24,25 Studies in animal models have identified several cellular mechanisms governing recruitment of progenitor cells to ischemic tissue.5 These
include intercellular adhesion molecule-1 (playing a crucial role in mediating progenitor cell recruitment to the infarcted myocardium), stromal cell-derived factor 1 (expressed in infarcted myocardium in direct proportion to the necrotic tissue and providing chemoattraction to the CXCR4-positive progenitor cells), or the high-mobility group box 1 protein (a nuclear protein released extracellularly in proportion to cell necrosis and modulating the affinity of progenitor cell integrins to their counterligands including intercellular adhesion molecule-1). Thus, our principal conclusion that infarct size determines the magnitude of progenitor cell uptake in the myocardial injury zone in humans is consistent with experimental observations on the mechanisms that regulate cell homing to ischemic tissue.

Clinical Determinants of CD34+ Cell Myocardial Uptake

The principal finding from the present study is that myocardial uptake of autologous CD34+ cells in humans is related to several different measures of infarct size (peak TnI, infarct size by SPECT, infarct border zone and total LGE mass by cMRI; Figure 4). This finding may explain apparent discrepancies within and between various previous studies on progenitor cell therapy in acute/recent MI in which patient recruitment and outcome analysis were based on the conventional parameter of early LVEF (at times assessed within 24 hours of STEMI and taken in the absence of a substantial myonecrotic marker release). Patient age and the number/severity of coronary artery disease risk factors have been shown to affect the functional properties of CD34+ cells. Despite the fact that the all-comer patient cohort in the present study was clearly heterogeneous with respect to these variables (Table), there was a significant correlation between the number of harvested cells and the total cell label activity after labeling ($r=0.72$, $P=0.007$). Our findings clearly indicate a lack of association between myocardial uptake of CD34+ cells and LVEF as determined by G-SPECT or by echocardiography on the day of cell delivery (day 7–12 after pPCI). Of note, the weak negative correlation between the magnitude of cell uptake and cMRI-determined LVEF may be related to the relative accuracy of MRI (versus echocardiography or G-SPECT) in LVEF assessment. Conversely, it is well known that LVEF may be a poor marker of recent infarct size because of postischemic myocardial stunning and early healing/remodeling, which has an influence on LVEF that is present for at least 2 to 4 weeks after AMI.

Recent pilot work by Porto et al suggested that endothelial progenitor cells might be mobilized from BM in proportion...
to the size of area at risk by T2-weighted cMRI rather than infarct size by LGE cMRI. In that study, however, cMRI was performed 2 to 4 days after pPCI (ie, when evidence indicates that retrospective evaluation of area at risk by quantifying the edematous tissue may be still adequate). This is in contrast to T2-weighted imaging in the second week of STEMI when there is a significant variation in edema volume in relation to the images at 12 to 48 hours or 5 to 7 days. Thus, T2-weighted cMRI acquisition at ≥7 days after STEMI (ie, as in the present study protocol; Figure 1) would be unlikely to provide any reliable retrospective evaluation of the area at risk. Moreover, several studies in large patient samples (note that the work by Porto et al involved 15 subjects) demonstrated a significant relationship between the size of area at risk and LGE imaged within 7 days of MI. In the present study, the timing of index cMRI image acquisition was deliberately set at 7 to 12 days (median, 10 days; Figure 1) after pPCI for several reasons. First, the complex study protocol did not accommodate early sequential cMRI imaging. Second, total LGE mass during the second week after infarct revascularization has previously been shown to quantitatively correlate with the amount of necrotic tissue in a highly reproducible fashion and to correlate with the long-term clinical outcomes better than LVEF. In addition, cMRI acquisition close to cell administration (Figure 1) enabled integrating infarct images with cell visualization (Figure 3). Although our timing of index cMRI image acquisition precluded any reliable retrospective determination of the area at risk, we used a validated algorithm to assess the infarct border zone size. Indeed, the mass of infarct border zone showed the strongest (of all considered infarct size parameters) correlation with the cell uptake (≈0.84, P<0.0001; Figure 4D), suggesting a central role for heavily ischemic (and only partly necrotic) tissue in progenitor cell recruitment.

Relevance of Cell Uptake in the Peri-Infarct Zone

In the present study, SPECT (and not MRI) was used for cell imaging because SPECT has a significantly greater sensitivity (≈105 cells with SPECT versus ≈105 cells with MRI). The 4-fold difference in resolution between SPECT and MRI and the fact that 3-dimensional SPECT is not quantitative for cell label imaging preclude any exact localization or quantification of the labeled cell signal in relation to MRI of the scar versus the heavily ischemic but viable tissue. In the future, this might be feasible with the use of novel, nanoparticle–based MRI labels. Although progenitor cells are known to migrate in the gradient of hypoxia-induced chemokines such as stromal cell–derived factor and although this gradient increases 4-fold from the border to the center of the infarct area, the highly hypoxic milieu in the infarct central zone is hostile to progenitor cells and may limit the functional significance of cell migration from the infarct border to the center (core) of the infarct zone. This suggests that any major early colonization of the infarct core zone that remains nonperfused at ≥7 to 10 days after pPCI is unlikely to occur after transcoronary cell delivery and thus limits the capacity of transcoronary-applied cells to affect the zone of irreversible myocardial injury.

The findings from the present study clearly indicate the key role of infarct border zone size in determining progenitor cell uptake (Figure 4D). Moreover, we demonstrated qualitative cell uptake in the peri-infarct zone by G-SPECT (hypoperfused area on the border of nonperfused tissue and normal myocardium; eg, in Figure 2B) and on the borders of cMRI-LGE zone (eg, in Figure 3, bottom). This finding is consistent with pilot data in animal models and humans that suggested that progenitor cell uptake might occur preferentially in the infarct border zone. The infarct border zone plays a central role in infarct expansion and cardiomyopathic LV remodeling because of the presence of myocytes susceptible to dedifferentiation and apoptosis. Thus, infarct border zone is a key target for therapies aimed at inhibiting adverse LV remodeling. Indeed, both experimental and clinical evidence suggests that failure to inhibit apoptosis and to stimulate the dedifferentiated surviving cells in the peri-infarct viable myocardium may be associated with adverse LV remodeling occurring despite surgical resection of the infarct scar. Further work is required to assess the impact of CD34+ cell uptake in the peri-infarct zone on the prevention of postinfarct LV remodeling and the role of cell-engineering strategies in boosting this effect.

Study Limitations

Migration and engraftment of CD34+ cells is an active and dynamic process whereas gadolinium can diffuse passively through damaged cardiomyocyte membranes. The use of a short-half-life radiotracer reduces the time frame during which cells can be imaged, a limitation inherent to our work. Long-term progenitor cell tracking with precise evaluation of migration zones is currently best achieved with gene assays, the use of which, however, is limited to animal models. The time to administer CD34+ cells in the present study (7–12 days after STEMI; Figure 1) was chosen to match the apparent maximal benefit of cell therapy in the REPAIR-AMI study and coincides with peak inflammatory response after MI. Although the present work suggests a biological mechanism between infarct size and cell uptake, whether (and to what extent) the distribution of transcoronary-delivered autologous CD34+ cells differs from what might be observed after labeled nonselected peripheral white blood cell infusion was not determined. In this regard, it is noteworthy that although both the CD34+ fraction and white blood cells appear to share several homing mechanisms, some of these mechanisms are preferential for the BM–harvested CD34+ progenitor cells.

Conclusions

In the largest study with labeled progenitor cells in humans with recent MI, we found that the early uptake of CD34+ cells delivered via the infarct-related artery 7 to 12 days after pPCI is determined by infarct size rather than by LVEF. This suggests that further studies of stem cell therapy after recent MI may need to consider objective measures of infarct size rather than the early LVEF as the patient inclusion criterion and study outcome parameter. The infarct size–cell uptake relationship identified in the present study is consistent with a biological role of CD34+ myocardial retention in the peri-infarct zone in patients with recent MI.
Acknowledgments
We thank Professor M.Z. Ratajczak, Stem Cell Institute, University of Louisville, KY, for his tutorials on the biology of progenitor cell trafficking. We thank Professor Michal Tendera of the Medical University of Silesia (Katowice, Poland) for his fundamental support from inception to execution of the study. We are grateful to Justyna Stefaniak of Data Management and Statistical Analysis (DMSA, Krakow, Poland) for data processing.

Sources of Funding
This work was supported by EU structural funds from the Innovative Economy Operational Program (POIG.01.02-00-109/09 Innovative Methods of Stem Cells Applications in Medicine); by the Ministry of Science and Higher Education, Poland (PBZ-KBN-0651/ P01/2007/32, PBZ-KBN-2422/P01/2007/32); and by the Heart Foundation in Krakow, Poland.

Disclosures
None.

References
CLINICAL PERSPECTIVE

Effective recruitment of progenitor cells to myocardial injury area is a prerequisite for therapeutic effect; however, determinants of cell uptake in humans with recent myocardial infarction have not been defined. Pivotal clinical studies of cell therapy in patients with recent myocardial infarction have used early left ventricular ejection fraction (usually ≤45%) as the main inclusion criterion and efficacy end point. This work identified, for the first time, a relationship between the uptake of transcoronary-delivered CD34+ cells in peri-infarct zone and infarct size determined by peak troponin I, number of left ventricular segments with severely abnormal/absent perfusion (single-photon emission computed tomography), and infarct border zone mass by cardiac magnetic resonance. In contrast, the degree of systolic function impairment at 7 to 10 days after ST-segment–elevation myocardial infarction was not a consistent determinant of cell uptake. Major implications of this work include the following: infarct size rather than left ventricular ejection fraction should be the primary nonclinical inclusion criterion and efficacy end point in myocardial cell therapy studies and future strategies using engineered cells should focus on boosting cell-mediated effects in the infarct border zone.
Infarct Size Determines Myocardial Uptake of CD34+ Cells in the Peri-Infarct Zone: Results From a Study of 99mTc-extametazime–Labeled Cell Visualization Integrated With Cardiac Magnetic Resonance Infarct Imaging

Piotr Musialek, Lukasz Tekieli, Magdalena Kostkiewicz, Tomasz Miszalski-Jamka, Piotr Klimeczek, Wojciech Mazur, Wojciech Szot, Marcin Majka, R. Pawel Banys, Danuta Jarocha, Zbigniew Walter, Maciej Krupinski, Piotr Pieniazek, Maria Olszowska, Krzysztof Zmudka, Mieczyslaw Pasowicz, Dean J. Kereiakes, Wieslawa Tracz, Piotr Podolec and Wojciech Wojakowski

_Circ Cardiovas Imaging_. 2013;6:320-328; originally published online December 27, 2012; doi: 10.1161/CIRCIMAGING.112.979633

_Circulation: Cardiovascular Imaging_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2012 American Heart Association, Inc. All rights reserved.

Print ISSN: 1941-9651. Online ISSN: 1942-0080

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

http://circimaging.ahajournals.org/content/6/2/320

Data Supplement (unedited) at:

http://circimaging.ahajournals.org/content/suppl/2012/12/27/CIRCIMAGING.112.979633.DC1

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Cardiovascular Imaging* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints:** Information about reprints can be found online at:

[http://www.lww.com/reprints](http://www.lww.com/reprints)

**Subscriptions:** Information about subscribing to *Circulation: Cardiovascular Imaging* is online at:

[http://circimaging.ahajournals.org//subscriptions/](http://circimaging.ahajournals.org//subscriptions/)
SUPPLEMENTAL MATERIAL

Infarct Size Determines Myocardial Uptake of CD34\(^+\) Cells in the Peri-Infarct Zone: Results from a Study of \(^{99m}\)Tc-extamatazime-labeled Cells Visualization Integrated with Cardiac Magnetic Resonance Infarct Imaging.

Running title: infarct size and CD34\(^+\) uptake

Piotr Musialek\(^{1,2}\), MD,DPhil, Lukasz Tekieli\(^{1,2}\), MD, Magdalena Kostkiewicz\(^{1,2}\), MD,PhD, Tomasz Miszalski-Jamka\(^2\), MD, Piotr Klimeczek\(^2\), MD, Wojciech Mazur\(^3\), MD, Wojciech Szot\(^2\), MD, Marcin Majka\(^4\), DSc,PhD, R. Pawel Banys\(^2\), MSc, Danuta Jarocha\(^6\), PhD, Zbigniew Walter\(^5\), MD, Maciej Krupinski\(^2\), MD, Piotr Pieniazek\(^{1,2}\), MD,PhD, Maria Olszowska\(^{1,2}\), MD,PhD, Krzysztof Zmudka\(^{2,6}\), MD,PhD, Mieczyslaw Pasowicz\(^2\), MD,PhD, Dean J. Kereiakes\(^3\), MD, Wieslawa Tracz\(^{1,2}\), MD,PhD, Piotr Podolec\(^{1,2}\), MD,PhD, Wojciech Wojakowski\(^7\), MD,PhD

\(^{1}\)Jagiellonian University Dept. of Cardiac & Vascular Diseases, Krakow, Poland
\(^{2}\)John Paul II Hospital, Krakow, Poland
\(^{3}\)The Christ Hospital Heart and Vascular Center – The Lindner Center for Research and Education, Cincinnati, Ohio, USA
\(^{4}\)Jagiellonian University Dept. of Transplantation, Krakow, Poland
\(^{5}\)Jagiellonian University Dept. of Hematology, Krakow, Poland
\(^{6}\)Jagiellonian University Dept. of Hemodynamics and Angiocardiography, Krakow, Poland
\(^{7}\)Third Division of Cardiology, Medical University of Silesia, Katowice, Poland

Corresponding Author
Piotr Musialek (MD DPhil)
Jagiellonian University Institute of Cardiology, Dept. of Cardiac and Vascular Diseases
John Paul II Hospital, Prądnicka 80, 31-202 Kraków, Poland
phone: +48126142287; fax: +484234376
email: pmusialek@szpitaljp2.krakow.pl
Example from a patient who would meet conventional criteria for inclusion in majority of previous progenitor cell therapy trials with an early post-MI cell-transfer protocol due to typical clinical presentation, typical EKG, and a significantly reduced ‘acute’ LVEF – but was excluded from the present study.

The images (Video 3, Video 4) show evolution of LVEF by catheter LV-graphy in a 56-year old patient with anterior STEMI (typical chest pain of 4h duration, ST segment elevation of 1mV in leads I and aVL, 2.5mV in V2-V4 and 1.5-2mV in V5-V6 in admission EKG). Prior to hospital admission the patient received from the paramedics team unfractionated heparin (5000 IU i.v.) and standard oral double antiplatelet therapy. There was a reperfusion arrhythmia when the patient was being installed on the catheterization table.

**Video 1.** LCA angiogram showing subtotal proximal LAD lesion with TIMI-2 flow, and a critical intermediate branch (IM) lesion with TIMI-3 flow.

**Video 2.** Final angiographic result of LAD-pPCI (stent 3.0x24mm) indicating an improved (though still impaired) flow in the infarct-related artery, and an optimal angiographic result of simultaneous stent-assisted IM-PCI.

**Video 3.** LV-gram directly after LAD-pPCI. Note akinesia of the mid anterior wall, apex and the adjacent segments. Global LVEF was 41%.

**Video 4.** Control LV-gram 7 days after LAD-pPCI. Note normal LV contractility with global LVEF of 68%. Peak troponin I was only 12.4ng/dL, consistent with lack of substantial myocardial tissue loss. A post-hoc diagnosis was made of myocardial stunning as the predominant mechanism of the early LVEF reduction.

Although the effect of stunning on LVEF is known to be present for to 2-4 weeks after STEMI (see Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E. Medical and cellular implications of...
stunning, hibernation, and preconditioning. *Circulation* 1998;97:1848-1867), most cell therapy studies to-date have recruited on the basis of early LVEF rather than infarct size – a strategy that may have confounded data in previous studies.