Five-year results of intracoronary infusion of the mobilized peripheral blood stem cells by granulocyte colony-stimulating factor in patients with myocardial infarction

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Aim
To evaluate the long-term effects of peripheral blood stem cell therapy in myocardial infarction (MI) patients.

Methods and results
A total of 163 patients with MI who were successfully revascularized with drug-eluting stents were enrolled and randomly assigned to four groups: acute MI (AMI) cell infusion, AMI control, old MI (OMI) cell infusion, and OMI control. We compared 5 years’ clinical outcomes between the cell infusion group (57 and 22 patients with AMI and OMI, respectively) and the control (60 and 24 patients with AMI and OMI, respectively). In the time-sequence comparison from baseline to 6 and 24 months follow-up after AMI, left ventricular ejection fraction (LVEF) by cardiac magnetic resonance imaging was significantly improved in the cell infusion group (n = 57), but not in the control group (n = 60). In the between-group comparison, the difference in improvement of LVEF for 2 years after AMI did not reach statistical significance between cell infusion and control groups. Intriguingly, the major adverse cardiac events for 5 years were significantly reduced in the cell infusion group (n = 79) compared with the control (n = 84; composite of cardiac death, non-fatal MI, hospitalization for heart failure and angina, and target vessel revascularization; 22.8 vs. 39.3%, P = 0.015).

Conclusions
Peripheral blood stem cell therapy has potential to improve long-term cardiovascular outcomes in MI patients.

Keywords
Myocardial infarction • Stem cell • Diabetes mellitus

Introduction
Recent clinical trials have reported various favourable effects of stem cell therapy in patients with acute myocardial infarction (AMI). Collectively, the results of these studies have indicated that stem cell therapy is feasible and safe, and improves left ventricular (LV) systolic function and myocardial perfusion. Most of these studies have been performed for short follow-up periods ranging from 3 to 24 months. Evaluation of long-term effects has been required, but only a few studies have reported long-term follow-up results until now.5,6

The key question of this study is whether effects shown in the short-term follow-up can maintain or change in the long-term follow-up. We think it is difficult that the infused stem/progenitor cell can survive up to several years, considering the limited long-term survival and low engraftment rate of the infused cells. However, clinical effects of stem/progenitor cell therapy were maintained up to 6–12 months in most short-term follow-up

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studies, when most of the infused stem/progenitor cells did not remain in the recipients’ hearts. We believe that it is possible that intervention in the certain phases of chronic progressive disease can favourably influence long-term outcomes. For example, in the long-term follow-up results of UKPDS, the intensive glucose control in the earlier phase of diabetes mellitus (DM) led to better clinical outcomes long after the randomized controlled study, generating the terminology of ‘legacy effect’ or ‘metabolic memory’. We did not know the long-term fates of stem/progenitor cell therapy in humans or whether there is a ‘legacy effect or cell-therapeutic memory’. We believe that long-term evaluation of the clinical influence of stem/progenitor cell infusion helps us to understand and improve stem/progenitor cell therapy.

To our knowledge, the long-term efficacy of intracoronary infusion of peripheral blood stem cells (PBSCs)/progenitor cells mobilized with granulocyte colony-stimulating factor (G-CSF) has not been reported before. Previously, we reported that intracoronary infusion of PBSCs improved LV systolic function during short-term follow-up.1,4,7 In the present study, we evaluated the long-term effects on LV systolic function and the clinical outcomes of G-CSF-based PBSC therapy during the 5-year follow-up.

Methods

This study is a long-term follow-up study for evaluation of pooled analysis of previously published MAGIC Cell-3-drug-eluting stents (DES)3 and its extension study. The MAGIC Cell-3-DES trial was a randomized, controlled clinical trial and complied with the Declaration of Helsinki. The Institutional Review Board of Seoul National University Hospital approved the study protocol. After explaining the procedure and potential risk of the study, we obtained informed written consent from the participating patients.

Patients and protocol

Patients with AMI and old myocardial infarction (OMI) were recruited between 1 January 2004 and 10 September 2006. The details of the study protocol of the MAGIC Cell-3-DES trial have been described previously.1 Additionally recruited patients for extension study also followed identical study protocol of previously reported MAGIC Cell-3-DES trial during randomization, treatments, and follow-up. Briefly, patients with MI who were successfully revascularized with DES were enrolled and randomly assigned to four groups: AMI cell infusion, AMI control, OMI cell infusion, and OMI control.

Exclusion criteria included the following: (i) persistent and severe heart failure [LV ejection fraction (LVEF) <20%]; (ii) uncontrolled myocardial ischaemia or ventricular tachycardia; (iii) culprit lesion of infarct-related artery not feasible for percutaneous coronary intervention (PCI) or unsuccessful PCI; (iv) age >80 years; (v) malignancy; (vi) serious current infection or haematologic disease; and (vii) life expectancy <1 year.

In this study, the objective was to evaluate the long-term effects of PBSC therapy on LV systolic function and the development of major adverse cardiac events (MACEs) in patients with MI, including cardiac death, MI, target vessel revascularization (TVR), or hospitalization due to aggravation of ischaemia or heart failure.

In the patients in the cell infusion groups, PBSCs were mobilized by daily subcutaneous injections of G-CSF (Dong-A Pharmaceutical, South Korea) at 10 μg/kg body weight for 3 consecutive days. On the fourth day, mobilized PBSCs were collected from patients with a COBE Spectra Apheresis System (Cobe BCT Inc., Lakewood, CO, USA) using the mononuclear cell collection method. We employed infused cell doses of 1–2 × 10⁷ mononuclear cells per patient to guarantee the minimum target-cell dose of 7 × 10⁶ CD34⁺ cells. For each patient in the cell infusion group, we selectively infused PBSCs into the infarcted myocardium via an over-the-wire balloon catheter, as described previously.8 No placebo was applied to the control group. During the entire follow-up period, all patients were managed according to usual current practices.

Clinical follow-up

Clinical follow-up visits were scheduled for at least every 6 months after the initial 6 month follow-up evaluation. Medical records were reviewed by the investigators and study coordinators. The investigators also asked patients about potential outcomes or adverse events at each visit.

Cardiac contrast-enhanced magnetic resonance imaging

Cardiac contrast-enhanced magnetic resonance imaging (CE-MRI) was performed after PCI at the baseline and 6 months follow-up, and for AMI patients, additionally at 24 months. Images were acquired during multiple breath-holds with a 1.5 T whole-body magnet (Sonata 1.5 T; Siemens, Germany). After localization of the heart, 8–11 contiguous short-axis slices of 8-mm-thick slices with a gap of 2 mm were acquired to cover the entire left ventricle from base to apex. The images of the heart were acquired at both end-systole and end-diastole. For LV-perfusion evaluation, we performed a dynamic MRI with a phase-contrast sequence. A 10-mL bolus of gadolinium-diethylene-triamine penta-acetate followed by a saline flush was administered to patients using an injection machine. Left ventricular ejection fraction and LV volumes were calculated using ARGUS (Siemens). We assessed regional LV function by determination of systolic wall motion with a 17-segment model, as proposed by the American Heart Association.6 Segmental wall thickening was assessed semi-quantitatively and judged visually, and scored to be: (i) normo-kinetic; (ii) hypo-kinetic; (iii) a-kinetic; or (iv) dys-kinetic. The wall motion-score index was defined as the sum of the scores for the 17 segments. The extent of segmental late enhancement was scored according to the following classification system: 0, 1–25, 26–50, 51–75, and 76–100% of either volume or trans-mural extent. Analysis of MRI data was performed by a blinded specialist.

Coronary angiography and quantitative coronary angiography

Coronary angiography was performed at both the initial PCI and at the 6-month follow-up. An independent blinded specialist performed quantitative coronary angiography with a CAAS Research 2.0.1 program (Pie Medical Imaging, Maastricht, the Netherlands). Revascularization procedures (PCI or bypass surgery) were defined either as target vessels or as non-TVR. Indications for revascularizations were at the discretion of the investigators (clinically driven revascularization).

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation and the data were compared using independent Student’s t-tests for inter-group comparisons and paired t-tests for intra-group comparisons. Categorical variables were expressed as percentages, and χ² or Fisher’s exact tests were used for comparison. For per-patient analysis, we included only the first event for each patient in the analysis. We
estimated time-dependent event rates by Kaplan–Meier survival curves, and determined P-values by performing log-rank statistics. For analysis of the follow-up studies, we assessed changes in functional and anatomical indices at follow-up by paired Student’s t-tests or one-way ANOVA. All statistical tests that we performed were two-sided, and values of \( P < 0.05 \) were considered significant. All analyses were performed with IBM SPSS, version 19.0 software (SPSS Inc., Chicago, IL, USA).

## Results

### Patient characteristics

A total of 163 patients were enrolled in this long-term follow-up study and randomized into four groups: 57 for AMI cell infusion group, 60 for AMI control, 22 for OMI cell infusion group, and 24 for OMI control group, respectively. These 163 patients were composed of the patients in the MAGIC Cell-3-DES trial \( (n = 102) \) whose short-term results were reported\(^1\) and additional AMI patients \( (n = 61) \) in the extension study.

Demographic, clinical, and angiographic baseline characteristics of the study participants were summarized in Table 1. There was no significant difference between the cell infusion and control groups in this study. There was no significant difference in baseline characteristics between AMI patients from the original MAGIC Cell-3-DES study and the extension study except for following two parameters (Supplementary material online, Table S1). History of previous MI \( (16 \text{ vs. } 51\% \; \text{P} \leq 0.001) \) and AMI caused by left anterior descending artery occlusion \( (48 \text{ vs. } 67\% \; \text{P} = 0.048) \) were more frequent in AMI patients from the extension study compared with AMI patients from the original MAGIC Cell-3-DES trial.

Mean \( 1.1 \pm 0.5 \times 10^5 \) mononuclear cells were infused in AMI cell infusion group \( (\text{CD34}^+ / \text{KDR}^+ : 9.0 \pm 12.4\% \), infusion volume: \( 6.3 \pm 2.6 \text{ mL} ) \) and mean \( 1.4 \pm 0.4 \times 10^9 \) mononuclear cells were infused in OMI cell infusion group \( (\text{CD34}^+ / \text{KDR}^+ : 2.3 \pm 12.4\% \), infusion volume: \( 8.7 \pm 3.8 \text{ mL} ) \).

### Left ventricular ejection fraction changes on cardiac contrast-enhanced magnetic resonance imaging in patients with acute myocardial infarction

At baseline, there were no significant differences between the AMI cell infusion group and the AMI control group in terms of LVEF \( (P = 0.630) \) and LV volume index \( (\text{LV end-diastolic volume: } P = 0.056) \). The cell infusion group showed significant improvement of LVEF at the 6- and 24-month follow-ups, respectively, compared

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td><strong>AMI Cell infusion ( (n = 57) )</strong></td>
</tr>
<tr>
<td><strong>Age, year</strong></td>
<td>57.5 ± 10.9</td>
</tr>
<tr>
<td><strong>Sex (male, %)</strong></td>
<td>84</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>24.7 ± 3.0</td>
</tr>
<tr>
<td><strong>Previous MI (%)</strong></td>
<td>32</td>
</tr>
<tr>
<td><strong>Risk factors (%)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>51</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>19</td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>47</td>
</tr>
<tr>
<td><strong>PCI situation</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Infarct-related artery (LAD/LCx:RCA)</strong></td>
<td>34:7:16</td>
</tr>
<tr>
<td><strong>Stent diameter (mm)</strong></td>
<td>3.09 ± 0.37</td>
</tr>
<tr>
<td><strong>Stent length (mm)</strong></td>
<td>26.8 ± 5.7</td>
</tr>
<tr>
<td><strong>Pain-to-balloon time (h), all/primary PCI</strong></td>
<td>69.7 ± 106.0/5.2 ± 2.7</td>
</tr>
<tr>
<td><strong>Primary PCI (%)</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>Medication (%)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Dual antiplatelets</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>ACEI/ARB</strong></td>
<td>86</td>
</tr>
<tr>
<td><strong>Beta-blocker</strong></td>
<td>75</td>
</tr>
<tr>
<td><strong>Statin</strong></td>
<td>70</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; OMI, old myocardial infarction; BMI, body mass index; MI, myocardial infarction; PCI, percutaneous coronary intervention; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; 1VD, 2VD, and 3VD, one vessel disease, two vessel disease, and three vessel disease, respectively; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.
with baseline [baseline vs. 6 month (n = 50): 51.7 ± 9.9 vs. 53.5 ± 9.4%, P = 0.44, baseline vs. 2 year (n = 42): 51.3 ± 9.4 vs. 54.1 ± 13.6%, P = 0.045], but the control group did not. Data from all patients and those from patients who completed follow-up are presented in Table 2. In the between-group comparison, the improvement of LVEF in the cell infusion group was mildly, but not significantly, greater than that in the control group at the 6- and the 24-months follow-ups (change of LVEF at 6 months: 2.5 ± 9.2 vs. 1.6 ± 10.6%, P = 0.666 and 2 years: 2.9 ± 9.2 vs. 1.7 ± 9.9%, P = 0.571), and there was no significant difference in changes of LV volume indices between the cell infusion and the control groups during follow-up.

There was no significant difference in LVEF and LV volume indices at baseline and follow-up between AMI patients from the original MAGIC Cell-3-DES group and the extension study. OMI patients were excluded for long-term CE-MRI analysis because a previously reported short-term study showed that there was no difference in change of LVEF between the cell infusion and the control groups at the 6-month follow-up.

**Clinical outcomes**

There was no serious adverse reaction related to G-CSF administration. In addition, there were no procedure-related serious adverse reactions during apheresis or cell infusion. A clinical follow-up was carried out for all the enrolled patients (Figure 1).

The mean follow-up duration was 55.2 ± 17.8 months. The occurrences of MACEs were significantly reduced in the cell infusion group compared with the control during follow-up (22.8 vs. 39.3%, log-rank P = 0.015, Figure 2, Table 3). The majority of clinical benefits in the cell infusion group came from reduction of TVR (17.7 vs. 33.3%, log-rank P = 0.014). Composite of cardiac death and MI was also less frequent in the cell infusion group but the difference was not significant (2.5 vs. 6.0%, log-rank P = 0.279). There was no late stent thrombosis during follow-up. There was no significant difference in medication status during follow-up between two groups.

In original MAGIC Cell-3-DES group, the occurrences of MACEs were significantly reduced in the cell infusion group compared with the control during follow-up (24.5 vs. 49.1%, log-rank P = 0.012). There was no significant difference in clinical outcomes between the original MAGIC Cell-3-DES group and the extension study group.

Benefits of stem cell therapy were more evident in diabetic patients (MACEs in the cell infusion group vs. control: 16.7 vs. 58.3%, log-rank P = 0.006) than non-diabetic patients (24.6 vs. 31.7%, log-rank P = 0.311). There was a significant interaction between stem cell therapy and DM in evaluation for the occurrence of MACEs (interaction P = 0.035: subgroup analysis Supplementary material online, Figure S1).

**Discussion**

**Influence of stem cell therapy on global LV function in AMI patients**

In this study, we found that, in the cell infusion group, LV systolic function significantly improved at 6 months after AMI compared with baseline and that this improvement persisted until the 24-month follow-up. However, the improvement of LVEF in the cell infusion group was not significantly greater than that of the control group. Considering this, clinical implications of the improvement of LVEF in the cell infusion group need to be evaluated in the larger long-term follow-up study.
For evaluation of LVEF, heterogeneity between the original trial and the extension study may need consideration. There was no significant difference between patients from the original trial and the extension study in changes of LVEF at 6 and 24 months and clinical outcome at 5 years. But, the direction of temporal changes in LVEF for 6 to 24 months in AMI patients receiving cell infusion were different, although statistically insignificant, between original and extension study; greater improvement at 6 months in original trial whereas at 24 months in extension study (changes of LVEF at 6 month in original vs. extension study: cell infusion group: $4.4 \pm 9.2$ vs. $0.5 \pm 6.5\%$, $P = 0.09$, control group: $0.7 \pm 9.2$ vs. $2.9 \pm 12.6\%$, $P = 0.541$; changes of LVEF at 24 month in original vs. extension study: cell infusion group: $2.2 \pm 9.0$ vs. $3.7 \pm 9.6\%$, $P = 0.541$, control group: $1.1 \pm 8.9$ vs. $2.3 \pm 11.3\%$, $P = 0.541$). Although there was no statistically significant interaction between temporal change of LVEF at 6–24 months and the data source whether from original or extension study, this may be considered as a limitation of this pooled analysis.

The follow-up loss can be considered as a major limitation of CE-MRI evaluation. Considerable numbers of patients did not participate in CE-MRI evaluation and follow-up loss can be considered as a confounding factor that influences the results. However, there were no significant differences in baseline characteristics and MRI indices between patients who completed CE-MRI follow-up and who did not. In the AMI control group, the clinical courses were quite similar between patients who did not complete CE-MRI evaluation and who did (MACEs: 24.2 vs. 25.9%, log-rank $P = 0.888$). However, in the AMI cell infusion group, patients who declined follow-up CE-MRI showed better clinical outcomes (MACEs: 0 vs. 27.9%, log-rank $P = 0.045$) than patients who completed evaluation.

**Clinical outcomes of stem cell therapy in the 5-year follow-up**

In this long-term follow-up, the patients in the cell infusion group showed better clinical outcomes than the control group. Although this study was underpowered to evaluate effects of PBSC therapy on clinical outcomes, it suggested that PBSC therapy has a potential to reduce cardiovascular events over control, which persisted during the 5-year follow-up. Interestingly stem cell therapy showed marked cardiovascular risk reduction compared with the control, even though improvement of LVEF was not so great. Effects of stem cell therapy on reducing cardiovascular events were consistent with previous reports. The REPAIR-AMI trial showed only a small improvement of LVEF, but bone marrow cell therapy significantly reduced cardiovascular events. Meta-analysis also reported similar results. This might suggest that LVEF may not be a good
surrogate to evaluate efficacy of current stem cell therapy in MI patients with preserved LV systolic function.

In this study, most of cardiovascular event reduction was derived from reduction of TVR. Baseline angiographic and interventional parameters were not significantly different between the cell infusion and the control groups (Supplementary material online, Table S2). Because the decision of repeated intervention was determined by the investigators’ own preference, open label design may cause bias. However, we did not believe that risk reduction with PBSC therapy was significantly influenced by bias. The differences in the TVR rate between the cell infusion and the control groups consistently diverged during the 5-year follow-up after completion of scheduled angiographic follow-up. Composite of cardiac death and recurrent MI was lower in the cell infusion group although it is not significant and the number of events is small. Its relative risk reduction was comparable with overall MACE reduction.

Several mechanisms can be suggested to explain long-term cardiovascular protective effects of PBSC therapy. First, improvement of LV systolic function and protection against LV remodelling can be a plausible explanation. Reduction of infarct size was evident at the 6-month follow-up. And improvement of LV synchrony also can lead to the improvement of myocardial systolic performance. Although these effects attenuated during follow-up, legacy effects could be expected. Intervention in early phase chronic progressive disease may delay disease progression during prolonged period and improve the clinical outcome such as a case of angiotensin converting enzyme inhibitor in AMI. Secondly, angiogenesis and improved myocardial perfusion can be considered. Lots of animal and clinical studies showed that PBSC mobilized by G-CSF can improve myocardial perfusion and reduce angina. Thirdly, vascular protective effects of PBSC therapy can be considered. We used G-CSF injection and combined PBSC intracoronary infusion. Both G-CSF and PBSC local infusion can accelerate endothelial healing, prevent atherosclerotic plaque progression, and improve endothelial function. Actually in this study, the late loss of the stented segment in AMI patients was less in cell infusion group than in control (Supplementary material online, Table S2), suggesting that cell therapy may enhance the endothelial recovery at the stented segment leading to less late loss and less TVR. Recent study has reported that single intracoronary infusion of bone marrow cells can enhance mobilization of circulating progenitor cells and effects sustained until the 1-year follow-up. The number of circulating endothelial progenitor cells was known to be predictor of cardiovascular events. Although the mechanism is unclear, this observation can be considered as one of the explanation for prolonged vascular protective effects by stem/progenitor cell therapy. However, sustainability of the beneficial effects of stem cell therapy on the coronary artery needs to be evaluated and explained by further investigation.

### Interaction between diabetes mellitus and stem/progenitor cell therapy

Potential dysfunction of autologous adult stem cell has been considered as an inherent limitation of adult stem cell therapy. Diabetes mellitus is an important risk factor for ischaemic heart diseases and unfavourably influences prognosis of MI. High-risk MI patients such as patients with DM can be good candidates for stem cell therapy. However, DM itself may reduce numbers and impair the functions of circulating stem/progenitor cells. This may limit the efficacy of stem cell therapy in DM patients with MI.

Although there have been concerns about dysfunction of stem/progenitor cell and efficacy of stem cell therapy in diabetic patients, in our study, PBSC therapy significantly reduced cardiovascular events in DM patients. There were no significant differences in number and composition of mobilized stem/progenitor cells by G-CSF between DM and non-DM patients. Unfortunately, we were not able to compare functionality of mobilized stem/progenitor cells in diabetic vs. non-diabetic patients. Based on our data, vasculo-protective effects of PBSC therapy in DM patients were well demonstrated. In our study, most of cardiovascular events were vascular events rather than myocardial events, and most patients had a relatively preserved LV systolic function. In this condition, vascular protective effects of PBSC might play a key role in

### Table 3 Major adverse cardiovascular events during 5 years follow-up

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Acute myocardial infarction</th>
<th>Old myocardial infarction</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cell infusion</td>
<td>Control</td>
<td>Cell infusion</td>
</tr>
<tr>
<td></td>
<td>(n = 79)</td>
<td>(n = 84)</td>
<td>(n = 57)</td>
</tr>
<tr>
<td>Composition of cardiac death, repeated MI, re-hospitalization due to HF or ischaemia, and target vessel revascularization: n (%)</td>
<td>18 (23)</td>
<td>33 (39)</td>
<td>0.015</td>
</tr>
<tr>
<td>Cardiac death: n (%)</td>
<td>1 (1)</td>
<td>4 (5)</td>
<td>0.193</td>
</tr>
<tr>
<td>Non-fatal myocardial infarction: n (%)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac death + non-fatal myocardial infarction: n (%)</td>
<td>2 (3)</td>
<td>5 (6)</td>
<td>0.276</td>
</tr>
<tr>
<td>Hospitalization due to heart failure: n (%)</td>
<td>2 (3)</td>
<td>5 (6)</td>
<td>0.263</td>
</tr>
<tr>
<td>Target vessel revascularization: n (%)</td>
<td>14 (18)</td>
<td>28 (33)</td>
<td>0.014</td>
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</tbody>
</table>
reducing cardiovascular events in DM patients. Although there might be biological dysfunction of stem/progenitor cells to some degree in DM patients, clinical efficacy of stem/progenitor cell therapy in DM patients might be sufficient to make them suitable candidates for cell therapy.

Limitations
First, this is a small study and underpowered to evaluate effects on clinical outcomes. However, we believe that, considering the nature of studies for evaluation of novel therapeutics, long-term complete clinical follow-up of a small study also can provide valuable insight into stem cell therapy.

Secondly, this study is a pooled analysis of two studies, in which the same protocol and treatment were applied. Pooled analysis of a heterogeneous population may reduce distortion of results. However, most current stem cell therapies can evaluate small numbers of patients and this is a major limitation of most stem cell therapies. In order to increase statistical power, we had to pool the data from two studies. The extension study was performed by the same investigators with the same protocol as original MAGIC Cell-3-DES trial. The baseline characteristics and status of cell infusion were similar between two studies and clinical courses and change in CE-MRI data were also similar between the patients from MAGIC Cell-3-DES and the extension study.

Thirdly, follow-up loss for evaluation of LV function can be a limitation. However, as discussed earlier, we believe that follow-up loss may not significantly influence the results, and we did not lose a single patient in clinical follow-up.

Lastly, the lack of core laboratory evaluation can be considered. However, we performed analysis of MRI data by a blinded specialist to avoid bias.

Conclusion
PBSC therapy with G-CSF improved the LV systolic function in AMI patients, which maintained until the 24-month follow-up. In addition, during the 5-year follow-up, stem cell infusion was associated with significantly reduced cardiovascular events, even in DM patients. However, the current study was underpowered to evaluate effect on clinical outcome and thus larger long-term follow-up study is required.

Supplementary material
Supplementary material is available at European Heart Journal online.

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Conflict of interest: none declared.

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