G-CSF for Extensive STEMI: Results from the STEM-AMI OUTCOME CMR Substudy

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早期治療G-CSF對於ST上升型心肌梗塞後的左心室功能障礙病人而言，可以助於改善心臟收縮功能、不良重建、瘢痕縮小、全心室縱向應變等。

G-CSF for Extensive STEMI

Results From the STEM-AMI OUTCOME CMR Substudy

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Rationale: **In the exploratory Phase II STEM-AMI (Stem Cells Mobilization in Acute Myocardial Infarction) trial, we reported that early administration of G-CSF (granulocyte colony-stimulating factor), in patients with anterior ST-segment–elevation myocardial infarction and left ventricular (LV) dysfunction after successful percutaneous coronary intervention, had the potential to significantly attenuate LV adverse remodeling in the long-term.**

Objective: **The STEM-AMI OUTCOME CMR (Stem Cells Mobilization in Acute Myocardial Infarction Outcome Cardiac Magnetic Resonance) Substudy was adequately powered to evaluate, in a population showing LV ejection fraction ≤45% after percutaneous coronary intervention for extensive ST-segment–elevation myocardial infarction, the effects of early administration of G-CSF in terms of LV remodeling and function, infarct size assessed by late gadolinium enhancement, and myocardial strain.**

Methods and Results: **Within the Italian, multicenter, prospective, randomized, Phase III STEM-AMI OUTCOME trial, 161 ST-segment–elevation myocardial infarction patients were enrolled in the CMR Substudy and assigned to standard of care (SOC) plus G-CSF or SOC alone. In 119 patients (61 G-CSF and 58 SOC, respectively), CMR was available at baseline and 6-month follow-up. Paired imaging data were independently analyzed by 2 blinded experts in a core CMR lab. The 2 groups were similar for clinical characteristics, cardiovascular risk factors, and pharmacological treatment, except for a trend towards a larger infarct size and longer symptom-to-balloon time in G-CSF patients. ANCOVA showed that the improvement of LV ejection fraction from baseline to 6 months was 5.1% higher in G-CSF patients versus SOC (P=0.01); concurrently, there was a significant between-group difference of 6.7 mL/m^2 in the change of indexed LV end-systolic volume in favor of G-CSF group (P=0.02). Indexed late gadolinium enhancement significantly decreased in G-CSF group only (P=0.04). Moreover, over time improvement of global longitudinal strain was 2.4% higher in G-CSF patients versus SOC (P=0.04). Global circumferential strain significantly improved in G-CSF group only (P=0.006).**

Conclusions: **Early administration of G-CSF exerted a beneficial effect on top of SOC in patients with LV dysfunction after extensive ST-segment–elevation myocardial infarction in terms of global systolic function, adverse remodeling, scar size, and myocardial strain.**

Clinical Trial Registration: **URL: https://www.clinicaltrials.gov. Unique identifier: NCT01969890.**

Visual Overview: **An online visual overview is available for this article.(*Circ Res*. 2019;125:295-306. DOI: 10.1161/CIRCRESAHA.118.314617.)**

Key Words: granulocyte colony-stimulating factor ■ left ventricular remodeling ■ myocardial infarction ■ percutaneous coronary intervention ■ standard of care

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Novelty and Significance

What Is Known?

- Cytokine release in the acute postinfarction period is crucial for the response to injury and modulates myocardial tissue repair. Cytokine response is particularly evident in the injured myocardium after ST-segment-elevation myocardial infarction (STEMI), contributing to cardiac remodeling.
- At preclinical level, the bone marrow leukocytes-mobilizing cytokine G-CSF (granulocyte colony-stimulating factor) has been demonstrated to ameliorate cardiac function and scar size after myocardial infarction through both cell-mediated and cell-independent mechanisms.
- Results from randomized clinical trials were, however, inconclusive.

What New Information Does This Article Contribute?

- This article reports the largest prospective randomized study with cardiac magnetic resonance testing the cardioprotective effects of G-CSF in STEMI patients with left ventricular dysfunction (LV) following primary reperfusion at high-risk of LV remodeling.
- Early subcutaneous administration of 5 μg/kg G-CSF twice a day for 6 consecutive days exerted beneficial effects in terms of global systolic function, adverse remodeling, scar size, and myocardial strain.

The therapeutic modulation of cytokines in STEMI has the potential to improve infarct healing and LV remodeling. Previous randomized clinical trials in STEMI using the bone marrow-mobilizer G-CSF have enrolled patients mainly at low-risk, with preserved LV function, small volumes, low prevalence of anterior myocardial infarction, and unknown symptom-to-balloon time. No other study except the STEM-AMI OUTCOME CMR (Stem Cells Mobilization in Acute Myocardial Infarction Outcome Cardiac Magnetic Resonance) Substudy trial have included patients affected by extensive STEMI with symptom-to-balloon time ≥2 and ≤12 hours, LV ejection fraction ≤45% after successful revascularization, and receiving G-CSF within 12 hours after reperfusion. The main results of this study are that early administration of G-CSF in STEMI patients at high risk of LV adverse remodeling is safe and can confer robust postconditioning cardioprotection on top of gold standard therapy in terms of LV global function amelioration, LV dilation counteraction, scar size shrinkage, and myocardial strain improvement. This drug-repositioning study suggests that G-CSF may be considered a viable therapy in STEMI patients with high likelihood of LV remodeling. These results may also have important implications for translational research aimed to better elucidate mechanisms by which G-CSF exerts a salutary effect onto the ischemic myocardium.

Nonstandard Abbreviations and Acronyms

2D-GCS	2D global circumferential strain
2D-GLS	2D global longitudinal strain
BM	bone marrow
CABG	coronary artery bypass grafting
CK-MB	Creatine kinase-MB
CMR	cardiac magnetic resonance
G-CSF	granulocyte colony-stimulating factor
HR	heart rate
ITT	intention-to-treat
LGE	late gadolinium enhancement
LV	left ventricular
LVEDV	left ventricular end-diastolic volume
LVEDVI	left ventricular end-diastolic volume indexed
LVEF	left ventricular ejection fraction
LVESV	left ventricular end-systolic volume
LVESVI	left ventricular end-systolic volume indexed
MACCE	major adverse cardiac and cerebrovascular event
MI	myocardial infarction
M_{LGE}	mass of the segments with late gadolinium enhancement
M_{LV}	left ventricular mass
MVO	microvascular obstruction
PCI	primary coronary intervention
SOC	standard of care
STEMI	ST-segment–elevation myocardial infarction

Despite groundbreaking progresses in the treatment of ST-segment–elevation myocardial infarction (STEMI) achieved by early reperfusion with primary percutaneous coronary interventions (PCI), the occurrence of adverse left ventricular (LV) remodeling remains associated with worse clinical outcome.[1–4] Adjunctive etiologic therapies aimed to reduce the amount of cell death after STEMI are at present still lacking.[5]

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Regenerative therapies have been investigated on top of standard of care (SOC) for their potential to restore the damaged myocardium subsequent to ischemic injury.[6,7] Based on a consistent body of evidence showing that the bone marrow (BM) is a source of cells which interplays with post-infarction processes leading to scar formation and maturation,[8,9] clinical trials of intracoronary delivery[10–13] or cytokine mobilization[14–23] of BM-cells have been performed with the goal to boost such a physiological response to ischemia.

G-CSF (granulocyte colony-stimulating factor) is an endogenous hematopoietic cytokine, produced by monocytes, fibroblasts, and endothelial cells, that is involved in the mobilization of granulocytes, stem, and progenitor cells from the BM into the blood circulation.[24] In addition, G-CSF, binding with its receptor expressed by cardiomyocytes after an ischemic injury, directly activates prosurvival signaling.[25] Although G-CSF has been consistently confirmed in experimental animal models to act as a regenerative and cardioprotective drug after STEMI,[26,27] however, clinical studies testing this hypothesis in humans have generated conflicting results.[28,29]

In the Phase II exploratory STEM-AMI trial (Stem Cells Mobilization in Acute Myocardial Infarction),[30] we have previously showed by cardiac magnetic resonance (CMR) that, 6 months after STEMI, indexed LV end-diastolic volume (LVEDVI) and infarct size were significantly reduced in patients receiving G-CSF as compared with placebo. Such an

attenuation of adverse LV remodeling was further confirmed at 3-year follow-up.[31]

Prompted by these evidences, the nationwide, multicenter, randomized, controlled, Phase III STEM-AMI OUTCOME trial (Stem Cells Mobilization in Acute Myocardial Infarction Outcome)[32] was designed to test the premise that early G-CSF therapy (Filgrastim, Hexal, Holzkirchen, Germany) in high-risk patients with STEMI may favorably impact their long-term outcome, by decreasing mortality and cardiac-related morbidity. Within such a pivotal trial, aimed at enrolling 1530 patients to G-CSF versus SOC randomized 1:1 ratio, the STEM-AMI OUTCOME CMR Substudy has been carved out to consistently test, by means of an adequate sample size, the hypothesis that G-CSF can favorably impact on LV remodeling and function, infarct size, and myocardial strain.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design

The STEM-AMI OUTCOME CMR Substudy trial was a substudy of the STEM-AMI OUTCOME trial.[32] The study complied with the Declaration of Helsinki and was approved by the Italian Medicines Agency (Agenzia Italiana del Farmaco, AIFA) and the local Ethical Committees of participating Institutes.

The STEM-AMI OUTCOME trial was designed to randomize to G-CSF or SOC (in a 1:1 ratio) patients with a first anterior STEMI undergoing a successful primary PCI or PCI rescue between 2 and 12 hours after symptom onset (or within 24 hours if symptoms persist) and with evidence of LV dysfunction within 24 hours after revascularization (LV ejection fraction [LVEF] ≤45% on 2D transthoracic echocardiography).

Patients randomized to treatment received, within 24 hours after reperfusion, 5 µg/kg G-CSF subcutaneously twice a day for 6 consecutive days (from day 0 to day 5) and prematurely interrupted if the threshold value of 50.000 white blood cells was reached. This threshold was set as hyperleukocytosis stopping rule in the previous STEM-AMI and similar G-CSF studies in STEMI.[16,23]

The exclusion criteria were (1) previous anterior myocardial infarction (MI); (2) known previous LV dysfunction (LVEF <45%); (3) angiographic evidence of coronary anatomy not suitable for PCI, or needing coronary artery bypass grafting (CABG); and (4) previous history of coronary artery bypass grafting or PCI on left anterior descending artery within 6 months. Additional exclusion criteria for the CMR Substudy was a known contraindication to CMR.

The primary composite end point of the main study was a reduced occurrence of any of the following in the G-CSF group compared with the control group over 2-year of follow-up: all-cause death, recurrence of MI, or hospitalization due to heart failure.

In February 2015, the STEM-AMI OUTCOME trial was amended to increase recruitment rates. The amendment widened patient enrollment to all-comer first STEMI with evidence of LV dysfunction after successful primary PCI having symptoms-to-balloon time within 12 or 24 hours if symptoms persisted. The study was stopped in February 2016 for sponsor's decision after Data Safety Monitoring

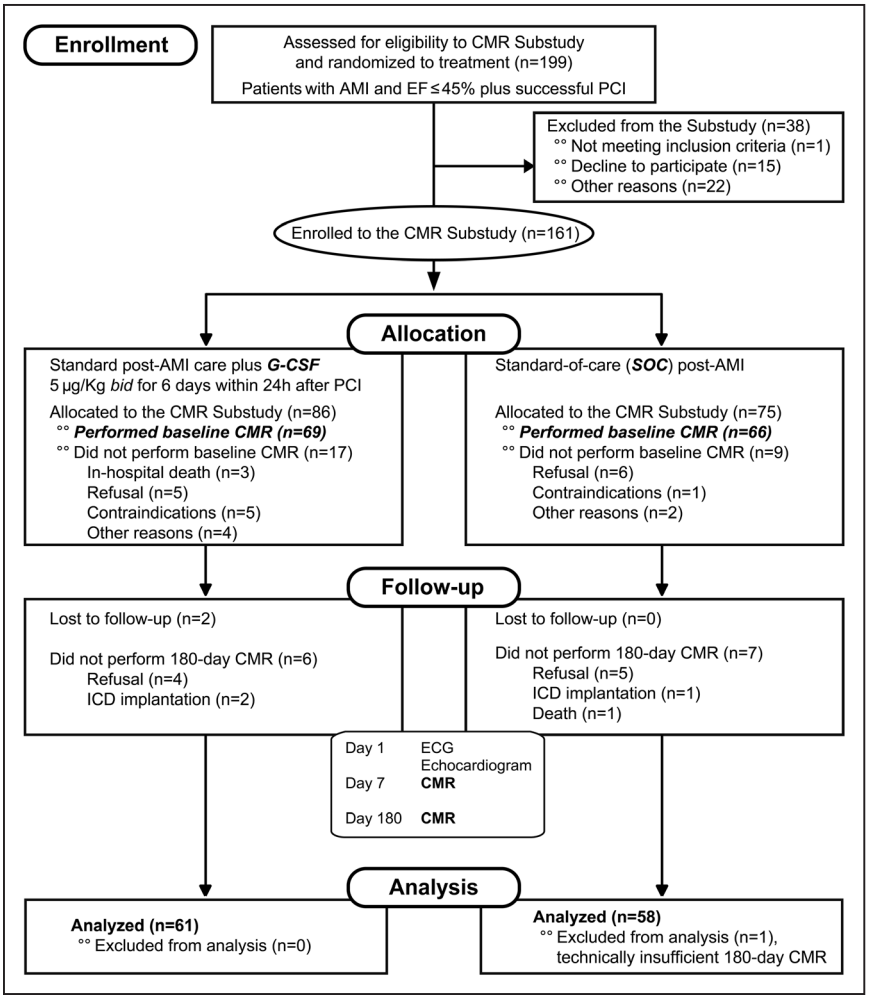

Figure 1. Flow chart of the STEM-AMI OUTCOME CMR (Stem Cells Mobilization in Acute Myocardial Infarction Outcome Cardiac Magnetic Resonance) Substudy. Patients were randomized 1:1 to G-CSF (granulocyte colony-stimulating factor) or to standard of care (SOC) and invited to participate in the CMR Substudy. Patients enrolled in the substudy, undergoing baseline CMR, were followed for 6 mo. CMR was repeated at follow-up. AMI indicates acute myocardial infarction; EF, ejection fraction; and PCI, primary coronary intervention.

Table 1. Demographic, Clinical, and Biochemical Patients' Characteristics

Variable	G-CSF (n=61)	SOC (n=58)	*P* Value
Age, y	60±10	62±10	0.44
Male sex, n (%)	50 (82.0)	49 (84.5)	0.81
Body mass index, kg/m^2	26.7±4.9	25.7±3.0	0.16
Cardiovascular risk factors			
Family history of CAD, n (%)	18 (29.5)	15 (25.9)	0.69
Current smokers, n (%)	30 (49.2)	26 (44.8)	0.71
Diabetes mellitus, n (%)	10 (16.4)	13 (22.4)	0.49
Hypertension, n (%)	17 (27.9)	21 (36.2)	0.43
Dyslipidemia, n (%)	19 (31.2)	24 (41.4)	0.26
Medical history			
Prior MI, n (%)	4 (6.6)	6 (10.3)	0.52
Prior TIA, n (%)	1 (1.6)	0 (0.0)	1.00
Angina, n (%)	4 (6.6)	4 (6.9)	1.00
CKD, n (%)	4 (6.6)	1 (1.7)	0.37
COPD, n (%)	3 (4.9)	1 (1.7)	0.62
PAD, n (%)	4 (6.6)	1 (1.7)	0.37
Blood analysis on admission			
Hemoglobin, g/dL	14.5±1.4	14.8±1.4	0.31
WBC count (×10^3/mm^3)	11.8±3.3	11.5±4.4	0.69
Platelet count (×10^3/mm^3)	235±57	236±59	0.96
Creatinine, mg/dL	0.92±0.33	0.93±0.23	0.88
Glycemia, mg/dL	149±45	159±57	0.27
C-reactive protein, mg/dL*	1.6±3.4	2.1±7.6	0.74
NT-proBNP, pg/mL†	1812±2082	1321±1347	0.27
Medication at discharge, n (%)			
Aspirin	60 (98.4)	57 (98.3)	1.00
Clopidogrel	4 (6.6)	5 (8.6)	0.74
Prasugrel	30 (49.2)	29 (50.0)	1.00
Ticagrelor	27 (44.3)	24 (41.4)	0.85
Statins	60 (98.4)	56 (96.6)	0.61
ACE inhibitors	56 (91.8)	51 (87.9)	0.55
Angiotensin receptor blockers	2 (3.3)	4 (6.9)	0.43
β-blockers	58 (95.1)	56 (96.6)	1.00
Diuretics	23 (37.7)	25 (43.1)	0.58

Categorical variables are presented as counts (n) and proportions (%); quantitative variables are expressed as mean±SD. ACE indicates angiotensin-converting enzyme; BNP, natriuretic peptide type B; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; G-CSF, granulocyte colony-stimulating factor; MI, myocardial infarction; PAD, peripheral artery disease; SOC, standard of care; TIA, transient ischemic attack; and WBC, white blood cell.

*n=48 and n=40 for the G-CSF and SOC groups, respectively.

†n=31 and n=28 for the G-CSF and SOC groups, respectively.

Table 2. Myocardial Infarction Related and Angiographic Characteristics

Variable	G-CSF (n=61)	SOC (n=58)	*P* Value
Cardiovascular condition			
HR	80.4±18.8	79.8±18.1	0.87
SBP, mm Hg	136.5±26.5	136.1±24.1	0.93
DBP, mm Hg	81.7±14.6	82.9±14.8	0.65
Killip class ≥3, n (%)	4 (6.6)	2 (3.4)	0.68
IABP, n (%)	3 (4.9)	2 (3.4)	1.00
ECG involved leads, n (%) Pts >5 leads	21 (34.4)	22 (37.9)	0.71
Echo LVEF, %	38.5±5.6	38.8±4.8	0.71
CK-MB peak, mg/L	254.5±178.2	201.0±155.6	0.08
Troponin peak, quintiles	3.1±1.5	2.9±1.4	0.40
MI site, n (%)			
Anterior	46 (75.4)	46 (79.3)	0.67
Inferior	4 (6.6)	1 (1.7)	0.37
Lateral	1 (1.6)	2 (3.4)	0.61
Inferior/lateral	2 (3.3)	2 (3.4)	1.00
Anterior/lateral	8 (13.1)	6 (10.3)	0.79
Anterior/inferior	0 (0.0)	1 (1.7)	0.49
Angiographic data			
Onset of MI to PCI, h	5.8±4.3	4.5±3.6	0.08
TIMI flow 0 pre-PCI, n (%)	44 (72.1)	40 (70.0)	0.84
TIMI flow 3 post-PCI, n (%)	58 (95.1)	56 (97.0)	1.00
PCI plus stent, n (%)	60 (98.4)	58 (100)	1.00
Drug eluting stent, n (%)	59 (96.7)	54 (93.1)	0.43
PCI rescue, n (%)	4 (6.5)	4 (6.8)	1.00
Multivessel disease, n (%)	32 (52.5)	31 (53.4)	1.00
PCI to G-CSF administration, min	784.6±501.7	…	…
Antithrombotic treatments, n (%)			
Aspirin	59 (96.7)	54 (93.1)	0.43
IIb/IIIa inhibitors	23 (37.7)	26 (44.8)	0.46
Clopidogrel	3 (4.9)	3 (5.2)	1.00
Prasugrel	29 (47.5)	29 (50.0)	0.86
Ticagrelor	26 (42.6)	24 (41.4)	1.00

Categorical variables are presented as counts (n) and proportions (%); quantitative variables are expressed as mean±SD. CK-MB indicates creatine kinase-myocardial band; DBP, diastolic blood pressure; G-CSF, granulocyte colony-stimulating factor; HR, heart rate; IABP, intraaortic balloon pump; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, primary coronary intervention; Pts, patients; SBP; systolic blood pressure; SOC, standard of care; and TIMI, thrombolysis in myocardial infarction.

Board recommendation due to low recruitment rate. Overall, 532 patients were recruited.

Twelve out of 44 participating Italian centers in the STEM-AMI OUTCOME trial joined the CMR Substudy. A complete list of CMR Substudy participating centers and investigators is available in Online Table I as well as the Steering Committee member list (Online Table II). Patients included in the STEM-AMI OUTCOME CMR Substudy were scheduled to perform CMR at 7 and 180 days after enrollment to assess LVEF, LVEDV, LV end-systolic volume (LVESV), infarct size, presence of microvascular obstruction (MVO) and myocardial strain.

Table 3. Changes Over Time of CMR-Measured Parameters Unadjusted and Adjusted for Baseline Variables

Δ_{T6-T0} in CMR Findings	Analysis	G-CSF	SOC	*P* Value
LVEF, %	Model 1	4.21 (1.45 to 6.97)	0.22 (–2.61 to 3.06)	0.0484
	Model 2	4.72 (1.94 to 7.50)	–0.32 (–3.17 to 2.53)	0.0153
	Model 3	4.46 (1.71 to 7.21)	–0.04 (–2.86 to 2.78)	0.0270
	Full model	4.75 (1.99 to 7.51)	–0.35 (–3.18 to 2.49)	0.0142
LVEDVI, mL/m^2	Model 1	0.93 (–3.11 to 4.97)	0.34 (–3.80 to 4.49)	0.8410
	Model 2	–0.10 (–4.11 to 3.91)	1.42 (–2.69 to 5.54)	0.6071
	Model 3	–0.11 (–4.04 to 3.82)	1.44 (–2.60 to 5.47)	0.5920
	Full model	–0.63 (–4.59 to 3.33)	1.98 (–2.08 to 6.05)	0.3747
LVESVI, mL/m^2	Model 1	–2.53 (–6.53 to 1.46)	0.92 (–3.18 to 5.01)	0.2347
	Model 2	–3.68 (–7.57 to 0.21)	2.12 (–1.88 to 6.11)	0.0456
	Model 3	–3.54 (–7.38 to 0.30)	1.97 (–1.97 to 5.91)	0.0522
	Full model	–4.12 (–7.94 to –0.29)	2.58 (–1.35 to 6.51)	0.0198
LGE (% of LV myocardial mass)	Model 1	–2.76 (–5.06 to –0.46)	–2.21 (–4.54 to 0.11)	0.7407
	Model 2	–3.07 (–5.42 to –0.73)	–1.90 (–4.27 to 0.47)	0.4945
	Model 3*	–2.87 (–5.19 to –0.54)	–2.10 (–4.45 to 0.24)	0.6487
	Full model*	–3.10 (–5.48 to –0.73)	–1.50 (–4.09 to 1.10)	0.3755
2D-GCS, %	Model 1	–2.93 (–4.85 to –1.00)	–1.53 (–3.50 to 0.45)	0.3161
	Model 2	–3.12 (–5.10 to –1.13)	–1.33 (–3.37 to 0.71)	0.2253
	Model 3	–3.06 (–5.05 to –1.06)	–1.51 (–3.68 to 0.65)	0.3032
	Full model	–3.31 (–5.36 to –1.26)	–1.34 (–3.55 to 0.86)	0.2071
2D-GLS, %	Model 1	–2.52 (–3.96 to –1.08)	–0.65 (–2.13 to 0.83)	0.0757
	Model 2	–2.62 (–4.11 to –1.13)	–0.54 (–2.07 to 0.99)	0.0616
	Model 3	–2.60 (–4.09 to –1.11)	–0.28 (–1.90 to 1.34)	0.0392
	Full model	–2.67 (–4.21 to –1.12)	–0.23 (–1.90 to 1.44)	0.0401

Data are expressed as mean (95% CI). Model 1 was the unadjusted analysis. Model 2 was adjusted for symptom-to-balloon time, CK-MB peak, and Killip score. Model 3 was adjusted for baseline M_{LGE}/M_{LV} and MVO. The full model was adjusted for symptom-to-balloon time, CK-MB peak, Killip score, baseline M_{LGE}/M_{LV}, and MVO. CK-MB indicates creatine kinase-MB; CMR, cardiac magnetic resonance; 2D-GCS, 2D global circumferential strain; 2D-GLS, 2D global longitudinal strain; LVEDVI, left ventricular end-diastolic volume indexed; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end-systolic volume indexed; M_{LGE}, mass of the segments with late gadolinium enhancement; M_{LV}, left ventricular mass; and MVO, microvascular obstruction.

*Baseline M_{LGE}/M_{LV} was not included in these models.

Based on the results of the phase II STEM-AMI trial,[30,31] the primary end point of the CMR Substudy was a LVEDV change of ≥10 mL/m^2 between baseline (day 7; ie, T0) and after 6 months (day 180; ie, T6). The secondary end points were a LVEF change >5% and infarct size reduction.[32] Safety end points were survival, major adverse cardiac and cerebrovascular events (MACCE), and rate of restenosis. MACCE were defined as death, reinfarction, heart failure, urgent revascularization, and stroke. Clinical events and MACCE were also recorded over the 6-month follow-up.

A main potential source of bias might have stemmed from the open design of this randomized controlled study. Adopted limitation strategies included: (1) CMR studies performed by experts blinded to treatment allocation; (2) imaging interpretation in a core laboratory, with blinded expert readers; and (3) adjudication of clinical events by an Event Validation Committee.

The reporting of this study was in agreement to the STROBE statement (Strengthening The Reporting of OBservational Studies in Epidemiology; Online Table III).[33]

Written informed consent was obtained for both the STEM-AMI OUTCOME trial and the CMR Substudy.

CMR Protocol and Analysis

A detailed description of the method is available in the Online Data Supplement.

Statistical Analysis

Details of statistical analysis are described in the Online Data Supplement.

Results

Baseline and Infarction-Related Characteristics

Centers participating in the CMR Substudy recruited and randomly assigned to G-CSF or SOC a total of 199 consecutive patients between November 2013 and December 2015. The flow of participants throughout the trial is detailed in Figure 1. Among the 161 patients who gave a written informed consent to the CMR Substudy, baseline CMR imaging was

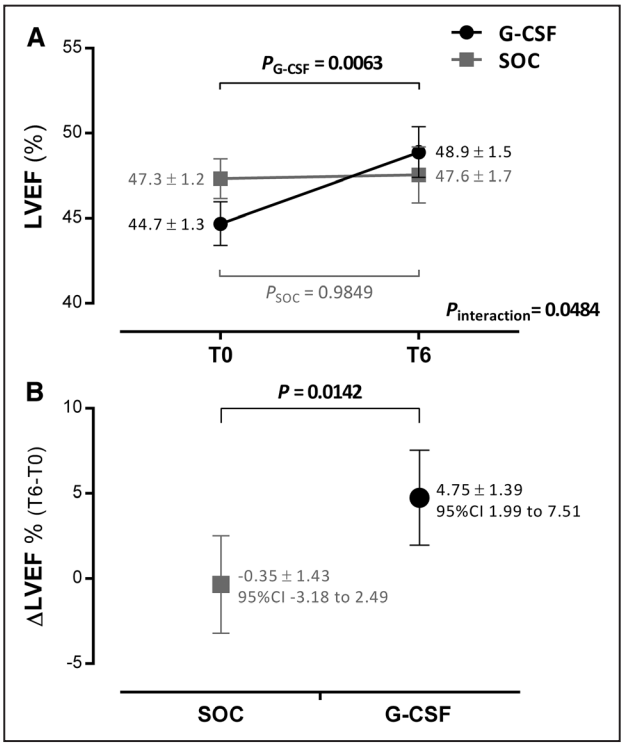

Figure 2. Changes in the left ventricular ejection fraction (LVEF) from baseline (T0) to 6 months (T6). **A**, LVEF increased significantly in the G-CSF (granulocyte colony-stimulating factor) group and not in the standard of care (SOC) group. Data are presented as mean±SEM. **B**, There was a significant difference in the Δ increment of LVEF over time (from T0–T6) in G-CSF vs SOC-treated patients, when adjusted for symptom-to-balloon time, creatine kinase-myocardial band (CK-MB) peak, Killip score, baseline M_{LGE}/M_{LV}, and microvascular obstruction (MVO). Data are presented as estimated marginal means±SEM and plotted with 95% CI. M_{LGE} indicates mass of the segments with late gadolinium enhancement; and M_{LV}, left ventricular mass.

obtained for 135 patients (n=69 G-CSF-treated subjects and n=66 controls). Fifteen patients (8 in the G-CSF and 7 in the control group) were lost at follow-up or did not perform the 6-month CMR, while one 6-month CMR imaging set in the G-CSF group was technically insufficient for analysis. Thus, paired imaging data sets were available for 119 patients (n=61 G-CSF-treated subjects and n=58 controls). Mean follow-up time was 6.3±1.4 months.

The demographic and clinical baseline characteristics and MI-related characteristics are listed in Table 1 and Table 2, respectively.

Ninety-two out of 119 patients (78%) had anterior MI. The 2 groups were similar for clinical features, cardiovascular risk factors, and medical therapy at discharge (Table 1). Moreover, as shown in Table 2, the 2D echocardiographic LVEF at enrollment was comparable in the 2 groups (38.5%±5.6 versus 38.8%±4.8 in the G-CSF and SOC groups, respectively). The G-CSF group exhibited a not-significant trend for a larger MI than the SOC group, as indexed by CK-MB (creatine kinase-myocardial band) peak levels, a worse Killip score, and longer symptom-to-balloon time.

G-CSF was administered an average of 13.1±8.4 hours after PCI. Patients received on average 7.7±3.3 administrations. According to the study protocol, G-CSF treatment was prematurely stopped in 41 patients who reached 50.000 white blood cell count. This latter group of patients received on average 7.1±3.0 G-CSF doses. No differences were observed in drug treatment at discharge between the 2 groups.

Clinical Outcome

Online Table V summarizes clinical events registered in hospital and during the 6-month follow-up in both treatment groups. We did not observe any MACCE or malignant arrhythmias during hospitalization, except for one urgent revascularization in the SOC group. Overall, a low incidence of nonfatal cardiac and noncardiovascular events over the 6-month observation period was recorded, with no significant differences between groups. Cumulatively, MACCE did not significantly change between the 2 groups at follow-up, even if their incidence was slightly higher among SOC with respect to G-CSF patients (10.3% versus 3.3%, respectively).

CMR Findings

Raw data of LVEF, LVEDV, and LVESV at baseline and follow-up for each G-CSF, and SOC patient are reported in Online Figure I.

Baseline and 6-month CMR quantitative measurements are listed in Online Table VI. Consistent with the CK-MB peak data, G-CSF patients showed on average a significantly larger infarct size than SOC subjects, as assessed by LGE mass, that is, the mean extent of M_{LGE}/M_{LV} was 4.5% greater in the G-CSF than in the SOC group (P=0.0224). This trend was maintained at 6-month follow-up (P=0.0515). Moreover, baseline MVO was more present, although not significantly, in G-CSF patients.

Functional Outcome

Changes over time of CMR-measured parameters are summarized in Table 3.

Data are presented unadjusted and adjusted for baseline variables that are independent predictors of worse outcome and show an imbalance between G-CSF and SOC groups. Model 1 was the univariable unadjusted analysis. Model 2 was adjusted for clinical variables related to STEMI prognosis (symptom-to-balloon time, CK-MB peak, and Killip score). Model 3 was adjusted for CMR baseline variables associated with adverse LV remodeling and worse outcome (LGE expressed as percentage of LV myocardial mass, and MVO). The full model was adjusted for all of the abovementioned baseline variables.

The G-CSF group showed a significant increase in LVEF from baseline to 6 months (a 4.2% gain on average, P=0.0063; Figure 2A), whereas no appreciable change was observed in the SOC group, with a significant interaction between time and treatment (P=0.0484), that is, suggesting that the magnitude of the effect of time on LVEF recovery was dependent on treatment. Most importantly, a significant difference in the change over time of the LVEF between the G-CSF and the SOC group was confirmed in all Models of the ANCOVA (Table 3), stepwise adjusting for baseline clinical and CMR variables related to MI extent and severity (symptom-to-balloon time, CK-MB peak, Killip score, M_{LGE}/M_{LV}, and MVO). Indeed, $\Delta LVEF_{T6-T0}$ was 5.1% higher on average in the G-CSF than in the SOC group (P=0.0142; Figure 2B). Consistently,

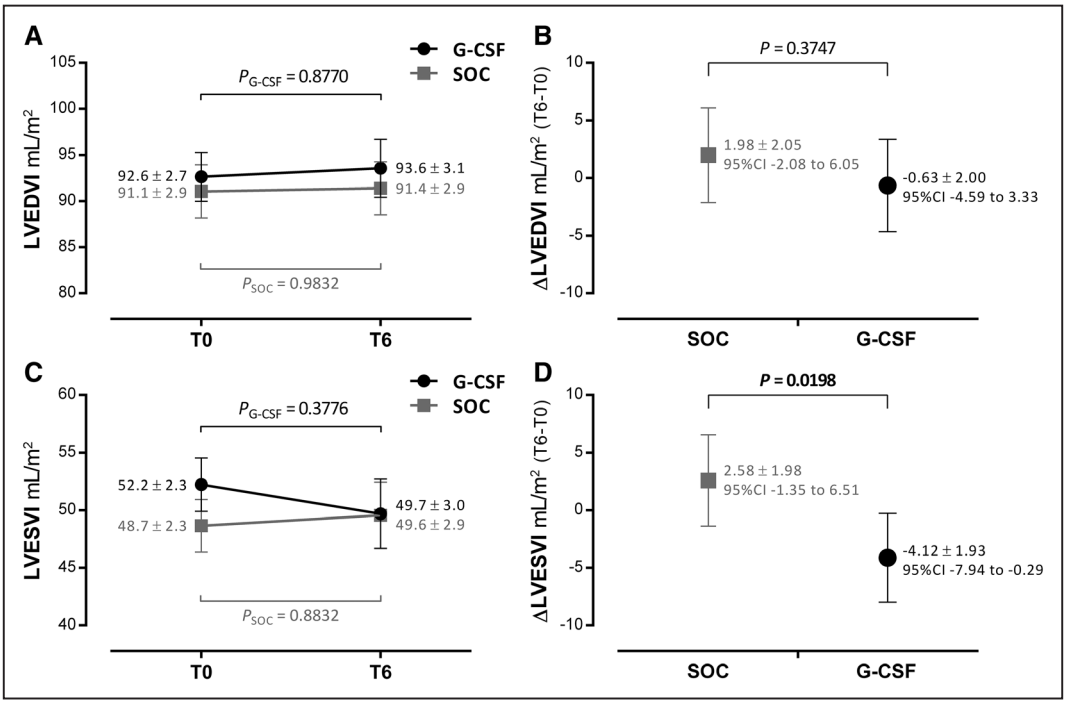

Figure 3. Changes in the indexed left ventricular end-diastolic (LVEDVI) and indexed left ventricular end-systolic (LVESVI) volumes from baseline (T0) to 6 months (T6). LVEDVI did not change over time in both the G-CSF (granulocyte colony-stimulating factor) and the standard of care (SOC) groups (**A**), whereas LVESVI showed a decreasing trend in the G-CSF group only (**C**). Data are presented as mean±SEM. When adjusted for symptom-to-balloon time, CK-MB (creatine kinase-MB) peak, Killip score, baseline M_{LGE}/M_{LV}, and microvascular obstruction (MVO), LVEDVI change over time (Δ_{T6-T0}) showed an increasing trend in the SOC group (**B**). On the contrary, there was a significant decrease in the Δ_{T6-T0} of LVESVI in G-CSF vs SOC-treated patients (**D**). Data are presented as estimated marginal means±SEM and plotted with 95% CI. M_{LGE} indicates mass of the segments with late gadolinium enhancement; and M_{LV}, left ventricular mass.

6-month LVEF was significantly higher in the G-CSF versus SOC group, after adjustment for the same baseline covariates (*P*=0.0446; Online Figure II). Finally, an analysis of the relationship between the symptom-to-balloon time and the change over time in LVEF showed that treatment with G-CSF resulted in a trend increase (*P*=0.075) towards the increase of the proportion of patients with LVEF improvement above the median value (2.5% in $\Delta LVEF_{T6-T0}$) when reperfused beyond 300 minutes (Online Figure III).

LVEDVI did not change in both groups from baseline to 6 months (Figure 3A). Consistently, the change over time of LVEDVI was not significantly different between G-CSF and SOC patients, even after adjustment for the abovementioned baseline variables (*P*=0.3747; Figure 3B), although a slight LVEDVI increase was present in SOC patients only. Similarly, LVESV indexed (LVESVI) did not significantly change in both groups from baseline to 6 months (Figure 3C), even if a decrease was observed in G-CSF patients only. However, covariance analysis showed that, when adjusting for baseline MI-related variables, there was a significant difference in the change of LVESVI from baseline to 6-month follow-up between the 2 groups: G-CSF patients tended to reduce and SOC patients to increase mean LVESVI, which resulted in a mean difference of 6.7 mL/m² in $\Delta LVESVI_{T6-T0}$ (*P*=0.0198; Figure 3D).

The analysis of the change of LGE mass normalized to LV mass (M_{LGE}/M_{LV}) from baseline to follow-up in the 2 groups resulted in a significant reduction in M_{LGE}/M_{LV} over time in the G-CSF group only (*P*=0.0383; Figure 4A), while the SOC group did not change, with a significant effect of the treatment factor (*P*=0.0423). The change over time of indexed LGE mass was not significantly different between G-CSF and SOC patients, even after adjustment for baseline MI-related variables (*P*=0.3755; Figure 4B), although a reduction was evident in the G-CSF group only.

Myocardial Strain Outcome

We also compared temporal changes of 2D longitudinal and circumferential strains in G-CSF versus SOC patients. 2D-GLS significantly improved in both groups, with a more pronounced amelioration in G-CSF patients (*P*<0.0001 and *P*=0.0129 in the G-CSF and SOC groups, respectively; Figure 5A). Consistently, when adjusted for the aforementioned baseline MI-related variables, the Δ improvement over time was significantly higher in the G-CSF group (−2.44% on average in G-CSF versus SOC group, *P*=0.0401; Figure 5B). In analogy, 2D-GCS improved significantly in the G-CSF group only (*P*=0.0063; Figure 5C), although the difference in the change over time between the 2 groups did not reach statistical significance after adjustment for baseline MI-related features (*P*=0.2071; Figure 5D).

Discussion

The main finding of this study is that, in patients with LV dysfunction after successfully reperfused STEMI, G-CSF treatment is effective in improving LV performance, as shown by a significant increase of LVEF and 2D-GLS due to reduced scar size, as well as in attenuating adverse LV remodeling as

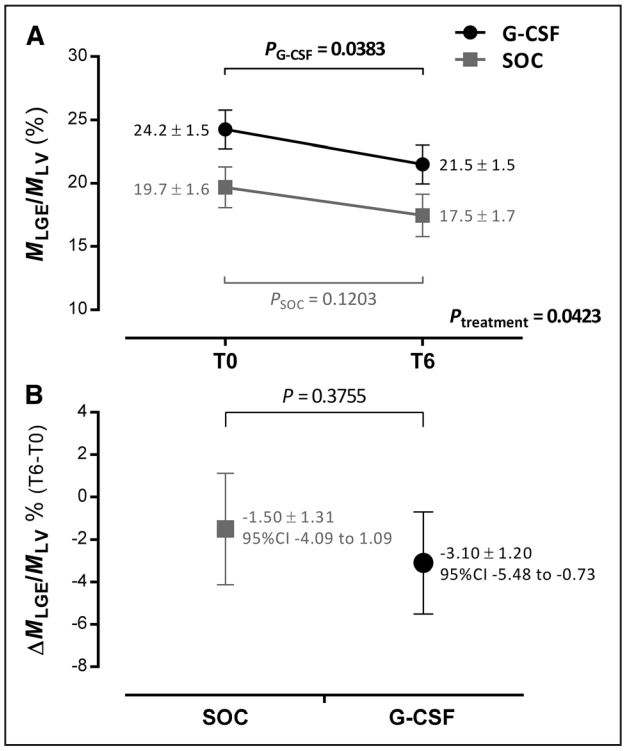

Figure 4. Changes in the mass of the segments with late gadolinium enhancement (M_{LGE}), normalized to the left ventricular mass (M_{LV}), from baseline (T0) to 6 months (T6). **A**, M_{LGE}/M_{LV} decreased significantly in the G-CSF (granulocyte colony-stimulating factor) group and not in the standard of care (SOC) group. Data are presented as mean±SEM. **B**, The Δ change over time in LGE showed a decreasing trend in G-CSF vs SOC-treated patients, when adjusted for symptom-to-balloon time, CK-MB (creatine kinase-MB) peak, Killip score, and baseline microvascular obstruction (MVO). Data are presented as estimated marginal means±SEM and plotted with 95% CI.

demonstrated by the significant reduction in LVESVI. To the best of our knowledge, we report here the largest study in STEMI investigating, by CMR, the cardioprotective and anti-remodeling benefits of early administration of G-CSF. These results corroborate the previous findings of the STEM-AMI trial, in which we have shown, in patients with a large anterior STEMI conditioning LV dysfunction receiving early G-CSF treatment versus placebo, an attenuation of LV remodeling in terms of adjusted LVEDVI at 6-month[30] and 3-year follow-up.[31]

The STEM-AMI OUTCOME CMR Substudy was conducted in the context of the larger STEM-AMI OUTCOME prospective, nationwide, multicenter, randomized, open-label, Phase III trial,[32] which was designed to randomize (1:1) 1530 STEMI patients to G-CSF versus SOC with the primary end point to assess whether G-CSF could reduce the occurrence of all-cause death, recurrence of MI or hospitalization due to heart failure. The sample size of the CMR Substudy (120 patients) was calculated according to the results of the previous STEM-AMI trial. Specifically, the prespecified primary end point was the change from baseline to 6 months of ≥10 mL/m^2 difference in adjusted LVEDV and as secondary end points a change of 5% in LVEF as well as infarct size reduction. Notably, the STEM-AMI trial was conceived more than a decade ago with an unique study design in the context of the contemporary published randomized trials testing G-CSF in STEMI.[30] At that time, the information available suggested that G-CSF appeared to be more salutary in STEMI in the presence of LV dysfunction and when initiated early[34] and that patients enrolled in previous negative trials with G-CSF were mainly at low-risk, with preserved LVEF (>50%), small volumes, and low percent anterior localization, and without definite symptom-to-balloon times.[20] No other study except the STEM-AMI trial had enrolled patients affected by anterior STEMI with a LVEF ≤45% after reperfusion with symptom-to-balloon time ≥2 and ≤12 hours.

Accordingly, the intention-to-treat (ITT) populations of the STEM-AMI OUTCOME trial and CMR Substudy have included patients with the same aforementioned features. Both studies have been, however, subsequently amended with the aim to increase accrual rate, thus shifting the initial ITT to a modified-ITT population to include all presenting STEMI patients showing LVEF <45% regardless of symptom-to-balloon time. The resulting changes have produced evenly differences between the ITT and modified-ITT populations in both G-CSF and control patients in terms of anterior STEMI frequency (from 100% to 75% and 79%, respectively). Although we do not have a clear explanation why in this study G-CSF counteracted adverse remodeling by acting on LVESV rather than, as prespecified, on LVEDV, however, modified-ITT changes may at least partly be taken into account for this difference. Nevertheless, since CMR is considered the gold standard technique for LV volumes and function evaluation, the significant 6.7 mL intergroup difference between G-CSF and SOC in adjusted LVESVI, which is a well-known independent prognosticator after STEMI,[35] is a thorough confirmation of the anti-remodeling effect exerted by G-CSF. Furthermore, the secondary study end points of LVEF improvement and infarct size reduction when adjusted for LV mass[36,37] have been matched. In fact, our findings showed that as compared with SOC, only G-CSF administration was associated with a significant reduction of LGE amount between baseline and follow-up timeline. As a result, G-CSF-treated patients showed an improvement over SOC of LV performance as demonstrated by a significant increase of unadjusted and adjusted LVEF, which consistently matched scar and LVESVI changes when adjusted for main clinical and CMR baseline covariates. It is well known that infarct healing and attenuation of myocardial remodeling play a fundamental role in the functional recovery of the LV.[38] Notably, these findings accounted for the presence of worse infarct-related profile in G-CSF patients at baseline which, if uncontrolled-for, may represent a potential bias for result's interpretation. Interestingly, the salutary functional effect on LVEF appears to be more evident at tardive reperfusion times (>5 hours), paralleling previous similar findings we reported for LVEDV.[30] Taken together, these findings suggest that G-CSF-mediated effects on damaged myocardium after extensive STEMI has the potential to translate into an improved clinical outcome. It is worth noting that in previous pivotal pharmacological studies dramatically impacting clinical practice after MI, apparently modest changes in LVEF (<5%) have been associated to improved prognosis.[39–42]

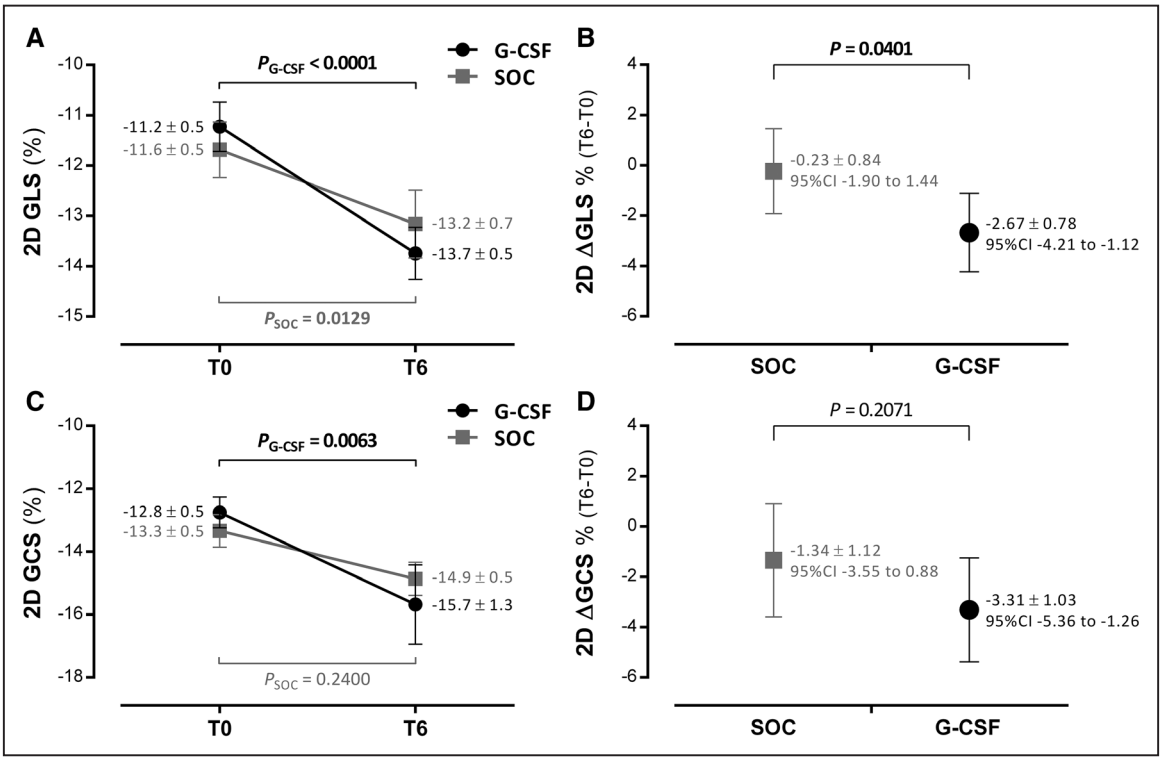

Figure 5. Changes in the 2-dimensional global longitudinal (2D-GLS) and 2-dimensional global circumferential (2D-GCS) strains from baseline (T0) to 6 months (T6). A–C, Both 2D-GLS and 2D-GCS decreased significantly more in the G-CSF (granulocyte colony-stimulating factor) with respect to the standard of care (SOC) group. Data are presented as mean±SEM. **B**, There was a significant difference between the Δ decrease over time of 2D-GLS in G-CSF (granulocyte colony-stimulating factor) vs SOC-treated patients, when adjusted for symptom-to-balloon time, CK-MB (creatine kinase-MB) peak, Killip score, baseline M_{LGE}/M_{LV}, and microvascular obstruction (MVO), whereas (**D**) only a trend in 2D-GCS. Data are presented as estimated marginal means±SEM and plotted with 95% CI. M_{LGE} indicates mass of the segments with late gadolinium enhancement; and M_{LV}, left ventricular mass.

Remarkably, in the present study, we further demonstrated for the first time a benefit of G-CSF on 2D-GLS, which is a well-established prognostic parameter in post-STEMI patients.[43] Feature tracking-CMR allows, with high spatial resolution, a retrospective tracking of LV myocardium based on cine images in both long- and short-axis views. Several studies demonstrated that abnormalities in future tracking-CMR-derived strain are linked to adverse remodeling.[44] Notably, in the largest study to date of patients with MI undergoing CMR with deformation imaging, Eitel et al[45] showed both 2D-GLS and 2D-GCS are associated with increased cardiac event rates after MI, whereas 2D-GLS had the strongest impact for the prediction of adverse prognosis at 1-year follow-up. Moreover, 2D-GLS is an independent predictor of clinical outcome after MI, even after adjustment for traditional cardiac risk factors including LVEF and infarct size.[45,46] Our data demonstrated, once adjusted for MI-related variables including LGE extent and presence of MVO, a significant amelioration of 2D-GLS in G-CSF-treated patients with respect to patients receiving SOC, as well as a significant 2D-GCS improvement in the G-CSF group only.

Previous studies with G-CSF after STEMI have generated mixed and confounding results. In particular, the STEMMI (Stem Cells in Myocardial Infarction) and REVIVAL-2 (Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells) trials, the only 2 studies evaluating post-STEMI effects of G-CSF using CMR as the imaging tool, have reported negative outcomes in terms of LV remodeling and function.[19,22] We believe, however, that some relevant differences have to be taken into an account to explain discrepancies with our data. Specifically, in these studies, patients were mainly at low-risk, with preserved LVEF, small volumes, and low prevalence of anterior MI and without unknown symptom-to-balloon time. In addition, in both trials, baseline LVEF at CMR was assessed before PCI, as reported in the majority of published cell therapy studies.[47] Conversely, in both the STEM-AMI trial and STEM-AMI OUTCOME CMR Substudy, we assessed baseline EF by CMR at day 7 post-PCI, when the myocardial stunning is partially resolved.[48] This aspect may partly explain the lack of EF improvement in the control group. We believe this feature as relevant for discriminating the effect of a new therapeutic agent on top of the well-known early benefit on ventricular function provided by PCI.[4] Furthermore, the STEMMI trial[19] was not powered to measure LVEF, and LV volumetric changes because the sample size was calculated on a prespecified end point change in systolic wall thickening. Moreover, this study did not enroll patients with LV dysfunction. Similarly, in the REVIVAL-2 study,[22] the mean LVEF as detected by CMR was 50%, and infarct size as measured by nuclear test was the primary prespecified end point. It is also worth underlying that the greatest benefits of cardioprotective mechanisms are reached when LV dysfunction is present.[34,49,50] Accordingly, in a porcine STEMI model of ischemia-reperfusion, it has been shown that a LVEF cutoff <45% is important for G-CSF therapy to result in significantly lower detriment of LVEF compared

with controls.[51] Interestingly, within published G-CSF trials in STEMI, only the FIRSTLINE study (The Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor), which shares with our study early G-CSF administration (within 1 day after PCI) and baseline LVEF cutoff ≤45%, showed positive results on EF, although by means of 2D echocardiography assessment.[18]

Several mechanisms have been proposed for the salutary effect of G-CSF in the infarcted heart, including angiogenesis, direct protection of cardiomyocytes from apoptosis, and reduction of myocardial fibrosis.[26] In this regard, it is worth mentioning that our results are in agreement with the REPAIR-AMI (Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction) Magnetic Resonance Imaging substudy as obtained after intracoronary BM-cell delivery.[52] This evidence, however, has not been confirmed afterwards.[53,54] The 2 approaches substantially differ in timing of cell activity, assuming that the G-CSF-driven BM-cell mobilization effect into the peripheral blood, which last 5 days, can be viewed as a repeated dose strategy versus the single shot administration of intracoronary cell delivery.

Furthermore, beside cell-dependent mechanisms, G-CSF was consistently shown to exert an adjunctive direct protective action on ischemic cardiomyocytes by activating pro-survival pathways.[25] Importantly, this effect is mediated by the expression of the G-CSF receptor by injured cardiomyocytes, which has been shown to peak into the infarcted area within 24 hours from coronary ligation and decline over 1 week in a mouse model of MI.[55] Moreover, in a pig ischemia-reperfusion model, Beohar et al[56] have investigated early and delayed G-CSF administration, showing that early treatment immediately after MI decreased ventricular dilatation, while delayed treatment (5 days) had a deleterious effect on LV remodeling. Accordingly, we believe that the early G-CSF administration scheme (within 24 hours from PCI) we followed in both the STEM-AMI and STEM-AMI OUTCOME studies has been a critical factor driving the success of G-CSF therapy.

Limitations

As previously mentioned, the CMR Substudy, as part of the Phase III STEM-AMI OUTCOME trial, was not designed with a placebo arm. However, we believe the single-blind randomization design, taking advantage of 2 experts CMR readers showing high accordance, ensures the reliability of the results given the functional and not patient-related nature of end points measured. In addition, although a modified-ITT population has been included in the statistical analysis, we believe that the resulting noncritical deviations from the ITT population have not affected the original study design, which was conceived to recruit patients with reperfused extensive STEMI and moderate-to-severe LV dysfunction.

Conclusions

Overall, the results of the CMR Substudy confirmed the protective mode of action exerted by G-CSF as indicated by measurable and relevant functional readouts in the post-infarction myocardium. Thus, suggesting G-CSF may be considered a viable therapy in STEMI patients with a high likelihood of adverse LV remodeling. Although cell-dependent and independent mechanisms by which G-CSF interplays with myocardial ischemic injury have to be fully elucidated, our data support the concept that G-CSF can confer robust post-conditioning cardioprotection on top of gold standard therapy in terms of preservation of LV global function, scar size shrinkage, as well as the counteraction of LV adverse remodeling.

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Disclosures

None.

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