Effects of Transendocardial Delivery of Bone Marrow–Derived CD133+ Cells on Left Ventricle Perfusion and Function in Patients With Refractory Angina

Final Results of Randomized, Double-Blinded, Placebo-Controlled REGENT-VSEL Trial

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Rationale: **New therapies for refractory angina are needed.**

Objective: **Assessment of transendocardial delivery of bone marrow CD133+ cells in patients with refractory angina.**

Methods and Results: **Randomized, double-blinded, placebo-controlled trial enrolled 31 patients with recurrent Canadian Cardiovascular Society II–IV angina, despite optimal medical therapy, ≥1 myocardial segment with inducible ischemia in Tc-99m SPECT who underwent bone marrow biopsy and were allocated to cells (n=16) or placebo (n=15). Primary end point was absolute change in myocardial ischemia by SPECT. Secondary end points were left ventricular function and volumes by magnetic resonance imaging and angina severity. After 4 months, there were no significant differences in extent of inducible ischemia between groups (summed difference score mean [±SD]: 2.60 [2.6] versus 3.63 [3.6], *P*=0.52; total perfusion deficit: 3.60 [3.6] versus 5.01 [4.3], *P*=0.32; absolute changes of summed difference score: −1.38 [5.2] versus −0.73 [1.9], *P*=0.65; and total perfusion deficit: −1.33 [3.3] versus −2.19 [6.6], *P*=0.65). There was a significant reduction of left ventricular volumes (end-systolic volume: −4.3 [11.3] versus 7.4 [11.8], *P*=0.02; end-diastolic volume: −9.1 [14.9] versus 7.4 [15.8], *P*=0.02) and no significant change of left ventricular ejection fraction in the cell group. There was no difference in number of patients showing improvement of ≥1 Canadian Cardiovascular Society class after 1 (41.7% versus 58.3%; *P*=0.68), 4 (50% versus 33.3%; *P*=0.63), 6 (70% versus 50.0%; *P*=0.42), and 12 months (55.6% versus 81.8%; *P*=0.33) and use of nitrates after 12 months.**

Conclusion: **Transendocardial CD133+ cell therapy was safe. Study was underpowered to conclusively validate the efficacy, but it did not show a significant reduction of myocardial ischemia and angina versus placebo.**

Clinical Trial Registration: **URL: http://www.clinicaltrials.gov. Unique identifier: NCT01660581.**

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Key Words: angina pectoris ■ angiogenesis ■ bone marrow ■ cell therapy ■ stem cell

Significant progress in the medical and interventional treatment of patients with stable coronary artery disease (CAD) contributed to the substantial improvement of long-term prognosis.[1–3] On the contrary, as a consequence of population aging and increasing burden of comorbidities, the number of patients with complex coronary

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Novelty and Significance

What Is Known?

- Endomyocardial cell therapy using nonselected bone marrow–derived mononuclear cells improves angina and myocardial perfusion in patients with refractory angina.
- Selected CD133 cells is a population enriched for angiogenic cells, which could potentially improve the efficacy of endomyocardial cell therapy, but to date no placebo-controlled trial was undertaken.

What New Information Does This Article Contribute?

- Endomyocardial injection by NOGA targeted hibernating (viable and with abnormal movement) areas of myocardium corresponding to segments of reversible perfusion deficits in SPECT with adenosine.
- The procedural and long-term safety was excellent.
- After 4 months, there was no significant difference in myocardial perfusion measured by SPECT with adenosine and angina class at 4 months and 1 year; however, there was a decrease of left ventricle volumes and decrease of the nitrate doses.

Study was stopped before reaching planned number of 60 patients because of slow enrollment, so with 31 patients (16 in the active arm and 15 in placebo), it lacked sufficient statistical power to detect the effects of such therapy; therefore, large multicenter trials are warranted.

Nonstandard Abbreviations and Acronyms

BM	bone marrow
CAD	coronary artery disease
LVEF	left ventricle ejection fraction
MRI	magnetic resonance imaging
SDS	summed difference score

atherosclerosis not amenable to standard revascularization increases.[4] Contemporary data show that the prevalence of refractory angina in CAD population ranges from 1% to 4%, probably because of lack of a standardized definition.[5–7] Although survival of patient with refractory angina seems to have improved with optimal medical therapy, registries show mortality rates as high as 4% per year.[8] Despite the optimal medical therapy, these patients complained of persistent angina associated with frequent hospital readmissions and decreased quality of life.[9] To date, cell-based products applied by intracoronary route have been tested in acute coronary syndromes, chronic myocardial ischemia, and heart failure. However, its efficacy is limited partially because of low myocardial retention of cells.[10,11] Among emerging interventional approaches, direct transendocardial (TE) application of autologous or allogeneic bone marrow (BM)–derived stem and progenitor cells is proposed as an adjunctive treatment strategy in the patients with refractory angina.[11–13] It was confirmed that this method is safe and feasible and has a positive impact on quality of life, with reduction of angina symptoms. Also, TE-delivered BM-derived cells seem to improve left ventricle (LV) function and myocardial perfusion.[14–17] To date, there is no head-to-head comparison of different types of cells concerning their efficacy. Selected BM-derived CD133+ cells consist of hematopoietic stem cells and endothelial progenitor cells and are enriched for endothelial and cardiac markers.[18] The main objective of this study was to assess the effects of transendocardial NOGA-guided injection of autologous BM-derived CD133+ cells on the improvement of myocardial perfusion, LV function, and angina symptoms in patients with refractory angina.

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Methods

REGENT-VSEL is an investigator-initiated single-center, randomized, double-blind, placebo-controlled prospective study with imaging and clinical outcome measures. The study was coordinated by an independent contract research organization (CRO; Kraków Cardiovascular Research Institute, Kraków, Poland), which provided the randomization system, monitored the trial, and performed the statistical analysis. All investigators remained blinded as to the allocation of the individual patients until database closure at 12 months clinical follow-up. Safety reporting protocol was adhering to European Medicines Agency and Food and Drug Administration pharmacovigilance guidelines.

Patients

Patients with refractory stable angina despite optimal medical therapy and not amenable for further revascularization (failed chronic total occlusion attempts, occluded vein grafts, and diffuse small vessel disease) were enrolled.

Inclusion Criteria

(1) Stable angina in Canadian Cardiovascular Society (CCS) II-IV class, despite maximum pharmacotherapy for at least 2 weeks since last medications change; (2) presence of ≥1 myocardial segment with reversible ischemia in qualifying Tc-99m single-photon emission computed tomography (SPECT); (3) disqualified from further revascularization by independent Heart Team; (4) age >18 and <75 years, and (5) written informed consent.

Exclusion Criteria

(1) Acute coronary syndrome ≤6 months; (2) heart failure New York Heart Association III-IV class; (3) LV ejection fraction (LVEF) <35%; (4) contraindications to NOGA procedure (ventricular wall thickness <8 mm, intracardiac thrombus, severe aortic stenosis, LV aneurysm or severe peripheral artery disease precluding the vascular access); (5) previous implantation of cardioverter defibrillator or pacemaker; (6) history of malignancy; (7) active infection; (8) life expectancy <6 months; (9) bleeding diathesis; (10) renal insufficiency (GFR <30 mL/min per 1.73 m^2), and (11) pregnancy, lactation, or lack of effective contraception in women of childbearing potential.

The screening was based on angina symptoms according to the CCS classification and validated by stress test when applicable. After providing an informed consent patients underwent baseline SPECT imaging followed by magnetic resonance imaging (MRI) as shown in Figure 1. Study procedures are summarized in Online Table I.

End Points

Primary End Point

The primary efficacy end point for this study was the change of myocardial perfusion by 99mTc-MIBI SPECT at 4 months after cell/placebo injection.

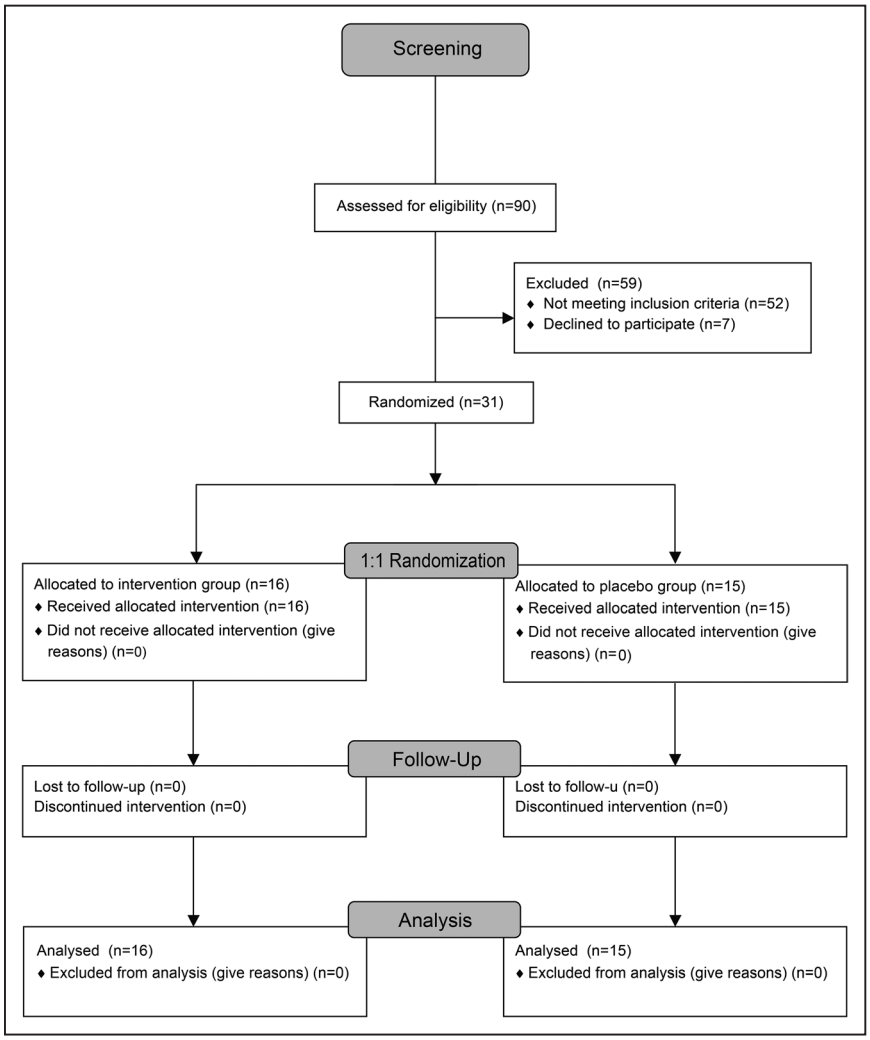

Figure 1. Patents disposition.

Secondary End Points

The secondary efficacy end points were (1) the change in LVEF, end-systolic (ESV) and end-diastolic volumes measured by MRI at 4 months after the procedure; (2) the change in the angina severity (CCS class) assessed after 1, 4, 6, and 12 months after the procedure.

Imaging

SPECT

A 1-day adenosine myocardial perfusion–gated SPECT study protocol was followed. Adenosine was infused intravenously at the rate of 140 µg/kg per min for 6 minutes. At the third minute of infusion, 370 MBq of 99mTc-MIBI was administered intravenously with continuous electrocardiographic and blood pressure monitoring. The same dose of 99mTc-MIBI was administered intravenously 6 hours later for the resting myocardial perfusion. 99mTc-MIBI-gated SPECT study was done 45 minutes after the administration of 99mTc-MIBI for both the adenosine stress and rest studies. The summed stress scores equaled the sum of the stress scores of all the segments, and the summed rest score equaled the sum of the resting scores of all the segments. The difference between the summed stress scores and summed rest score is summed difference score (SDS), and it was a measure of inducible ischemia.[19] The paired SPECT images were evaluated by 2 independent imaging specialists blinded to the allocation of treatment arm. A detailed description of SPECT protocols is in the Online Data Supplement.

Cardiac MRI

MRI studies were performed using a 1.5-T unit (Magnetom Avanto, Siemens) with magnetic gradient amplitude 40 mT, slew rate 200 mT/m per second, and a dedicated 4-element phase-array receiver coil. The examination protocol used for LV function consisted of TruFISP cine ECG-gated sequence in 2-chamber, 4-chamber, 3-chamber, and short-axis planes. LVEF, LV end-diastolic volume and ESV indexes, stroke volume, cardiac output, and LV mass were assessed using a dedicated workstation (Leonardo, Siemens), using the CMR imaging software (Argus, Siemens). A detailed description of MRI protocols is in the Online Data Supplement.

Cell Isolation

Approximately 150 mL of BM was aspirated from posterior iliac spines under conscious sedation. Harvested BM was purified and after preprocessing according to manufacturer's protocol incubated with anti-human CD133 antibodies conjugated with microbeads loaded onto the separation column (Miltenyi Biotec, GmbH). The positively selected cell fraction was suspended in 12 mL of 0.9% NaCl containing additional 10% donor serum. Hematology team performed randomization. Finally, cells/placebo was collected in identical syringes to maintain blinding of the cardiology team and transported to the catheterization laboratory. As a placebo, a cell-free control medium of 0.9% NaCl with the addition of 10% donor serum was used. A detailed description of cell isolation protocol is in the Online Data Supplement. Online Figure I shows the fluorescence-activated cell sorter analysis of isolated bone marrow cells.

Transendocardial Injection

Electromechanically guided transendocardial stem cells injection was performed using the NOGA-XP system (Biosense Webster, Johnson & Johnson, Diamond Bar, CA). The assessment of myocardial

viability allowed for the identification of hibernated areas of myocardium defined as a region with impaired mechanical and preserved electric activity (≥6.0 mV), correlating with reversible perfusion defects detected by the SPECT imaging. Stem cell injection was performed with the 8F MyoStar catheter. A detailed description of NOGA protocol is in the Online Data Supplement.

Clinical Follow-Up

Clinical evaluation and safety profile follows the schedule of CRO-monitored outpatient visits: 1, 4, 6, and 12 months after hospital discharge. Arrhythmia was evaluated with repeated 24-hour ECG monitoring.

Ethics

The study conforms to the Declaration of Helsinki. It was approved by the Ethic Committee of the Medical University of Silesia in Katowice and the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products and registered under no. NCT01660581 at www.clinicaltrials.org. All patients gave a written informed consent.

Statistical Analysis

All analyses were performed according to the intention-to-treat scheme. Categorical variables were presented as numbers and percentages. Continuous variables were expressed as mean±standard deviation (SD) or median and interquartile range. Differences between groups were compared using the Student's or the Welch's *t* test depending on the equality of variances for normally distributed variables. The Mann–Whitney *U* test was used for non-normally distributed continuous variables. Normality was assessed by the Shapiro–Wilk test. Equality of variances was assessed using the Levene's test. Categorical variables were compared by the Fisher exact test for 2×2 tables or by the Pearson's chi-squared test for other tables. For pairwise comparisons, the paired *t* test or the F-test was used if the difference between variables was normally distributed. The Wilcoxon signed-rank test was used for non-normally distributed differences. Paired categorical variables were compared by the McNemar–Bowker test. Two-sided *P* values <0.05 were considered statistically significant. Power calculation was based on our previous experience and data from literature,[17] as well as expected rate of patients enrollment in our department in 2010 where the study was approved. The initial assumption was a change of SDS between baseline and 4 months follow-up in active treatment: 2 and in the placebo group: 0.5, with SD=2, power=80%, alpha=0.05, and sampling ratio 1:1.

All calculations were done with JMP, Version 9.0.0 (SAS Institute Inc, Cary, NC), 1989 to 2007.

Table 1. Baseline Characteristics of the Study Groups

	Placebo Group (n=15)	Stem Cell Group (n=16)	*P* Value
Age, mean (SD), y	61.7 (5.4)	64.2 (7.2)	0.28
Man, n (%)	11 (73.3)	12 (75.0)	>0.99
Cardiovascular risk factors			
Smoking, n (%)	6 (40)	10 (62.5)	0.29
Hypertension, n (%)	13 (86.7)	15 (93.7)	0.60
Diabetes mellitus, n (%)	8 (53.3)	4 (25)	0.15
Chronic kidney disease, n (%)	2 (13.3)	1 (6.2)	0.60
Dyslipidemia, n (%)	14 (93.3)	15 (93.7)	>0.99
Family history of CAD, n (%)	5 (33.3)	8 (50)	0.47
Chronic medications			
Aspirin, n (%)	15 (100)	16 (100)	>0.99
Clopidogrel, n (%)	6 (40)	6 (37.5)	>0.99
ACE-I, n (%)	12 (80)	13 (81.2)	>0.99
ARB, n (%)	3 (20)	3 (18.7)	>0.99
Statins, n (%)	15 (100)	16 (100)	>0.99
Calcium channel blockers, n (%)	7 (46.7)	5 (31.2)	0.47
β-Blockers, n (%)	14 (93.3)	15 (93.7)	>0.99
Nitrates, n (%)	4 (26.7)	10 (62.5)	0.07
Diuretics, n (%)	10 (66.7)	6 (37.5)	0.15
Medical history			
Prior myocardial infarction, n (%)	11 (73.3)	10 (62.5)	0.70
Prior CABG, n (%)	12 (80)	13 (81.2)	>0.99
Prior PCI, n (%)	10 (66.67)	12 (75)	0.70

Smoking current or past; ACE-I indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; CABG, coronary artery bypass grafting; CAD, coronary artery disease; and PCI, percutaneous coronary intervention.

Results

Enrollment and Patients Allocation

Enrollment of 60 patients was initially planned. However, the study was stopped because of slow recruitment. Between July 2012 and September 2015, 90 patients were screened for inclusion. Eventually, 31 patients were enrolled and randomly assigned to active treatment arm receiving CD133+ cells (n=16) or placebo arm (n=15) as shown in Figure 1.

Baseline Characteristics

Table 1 shows the patient's baseline characteristics. The study population consisted of 23 (70%) males, and the mean age was 63.0 (±6.4) years. Approximately 60% had angina class III-IV, despite optimal medical therapy. Baseline (qualifying) SPECT revealed a mean summary stress score of 4.19 (±2.77). All of the patients had prior revascularization procedures, with 25 (80%) patients with a history of CABG and 22 (70%) of PCI. Twenty-one patients had a history of myocardial infarction (MI). CAD risk factors were highly prevalent, with hypercholesterolemia in 93%, diabetes mellitus in 38%, hypertension in 90%, and current or past smoking in 52% of patients. Comparison of the study groups showed no significant differences in baseline characteristics. Also, the medical therapy was comparable in both groups at baseline and remained unmodified throughout the study in all but 2 patients who presented with unstable angina within 4 months.

The use of nitrates was numerically lower in the active treatment arm (37.5% versus 73.3% versus placebo, respectively; *P*=0.07), but the difference was not significant.

BM Harvesting and CD133 Cell Isolation

In all patients, the BM (mean [±SD] volume of 160.0 [±20.7] in active treatment arm versus 153.1 [±12.5] mL in the placebo group; *P*=0.26) was aspirated without adverse events and transferred to hematology department. Mean cell processing time was 371.25 (±72.58) minutes. The mean volume of cells/placebo delivered for injection was comparable in both groups (*P*=0.87). The mean number of viable CD133 cells was $3.2\pm2.4\times10^6$.

Transendocardial Cell Injection

All 31 patients underwent endomyocardial mapping, with 200 to 250 valid points collected from the endocardial surface

and injections of cells/placebo. After processing of the maps, the region of interest has been chosen according to electromechanical parameters, consistent with viable myocardium with abnormal local linear shortening (hibernating areas). Cells/placebo were injected to region of interest according to previously published protocols (loop stability, the perpendicular orientation of the catheter, ventricular premature beat after needle insertion).[17] The median of 10 injections (min/max 8–16) of 200 μL were performed, and the mean volume of cells/placebo was comparable in both groups. In all patients, vascular closure device was used. All patients underwent transthoracic echocardiography on the day of injection or the following day, and no case of tamponade/pericardial effusion was noted. The differences in the number of injections were associated with procedural factors. Two patients (one from active and one from placebo groups) received lower than median of number of injections (8 and 9, respectively). The NOGA map showed separated small areas of hibernating myocardium located in the inferior wall, and the access to the area was compromised by chordae, so the number of injection was the maximum achievable. The dosing ranges were 2.8×10^6 and 5.3×10^6.

Primary End Point

Baseline and 4-month follow-up SPECT results were available in all patients. The baseline values of SDS were not significantly different between groups. The absolute difference of SDS between baseline and follow-up was larger in active treatment arm in comparison to placebo but did not reach statistical significance. Similarly, there were no significant differences in total perfusion deficit at baseline between placebo and cell-treated arm. After 4 months, there were no significant differences between placebo and active groups (mean [±SD], 3.6 [3.6] versus 5.0 [4.3]; P=0.32). During the 4-month follow-up, the SDS and SDS% were reduced in both groups, but the differences between baseline and follow-up were not significant. Summary of the SPECT findings are shown in Table 2. Figure 2 shows the individual changes in the SDS.

Table 2. Changes in the SPECT Parameters in 4-Months Follow-Up After Cell/Placebo Injection

	Placebo Group	Stem Cell Group	*P* Value*
Summed difference score			
Baseline	3.33 (1.9)	5.00 (3.2)	0.09
4-month follow-up	2.60 (2.6)	3.63 (3.6)	0.52
Change from baseline	−0.73 (1.9)	−1.38 (5.2)	0.65
P value†	0.17	0.30	
Total perfusion deficit, %			
Baseline	4.93 (2.7)	7.20 (4.7)	0.11
4-month follow-up	3.60 (3.6)	5.01 (4.3)	0.32
Change from baseline	−1.33 (3.3)	−2.19 (6.6)	0.65
P value†	0.19	0.20	

Data are mean (SD) unless otherwise stated. SPECT indicates single-photon emission computed tomography.

*P value of independent (unpaired) samples test.

†P value of dependent (paired) samples test.

In the active treatment group, there were more patients with a reduction of the number of ischemic segments than in the control group, but this difference was not statistically significant.

Factors Associated With Improvement of Ischemic Segments in SPECT

Logistic regression models were designed to evaluate the influence of several variables on improvement in SPECT—evidenced by reduction of inducible ischemia regions by at least one segment. Following factors were included into univariate logistic regression analysis: baseline number of ischemic segments (summed stress scores) after adenosine infusion, baseline LVEF (measured by MRI and SPECT), history of MI, and treatment arm (active treatment versus placebo). Because of the relatively small sample, multivariate regression analysis was not done. Table 3 shows the results of logistic regression models. In general, none of the factors were proved to be the independent predictor of reduction of ischemic area in SPECT; however, for the allocation to the active arm, the odds of positive response are 3.5× higher than the odds in the case of the assignment to the placebo arm. Also for the history of MI, the odds of positive response are 1.5× higher than in the case of negative MI history. Because 4 patient after core laboratory evaluation had <1 full segment of reversible ischemia, the per-protocol analysis was done, which revealed similar results as an intention-to-treat analysis with no significant improvement of perfusion after 4 months (Online Table II).

Secondary End Points

Paired MRI images were available in 26 patients. The paired MRI images were evaluated by an experienced independent radiologist blinded to the allocation to treatment. In 5 patients, the second MRI was not performed according to patients request (claustrophobia or anxiety during the first procedure). Baseline LVEF was comparable between the placebo and active treatment groups (mean±SD 53.6 [6.6] versus 48.5 [9.8]; P=0.13), whereas the LV volumes were significantly higher in the active treatment arm (LVESV 59.9 [15.2] versus 83.4 [32.7], P=0.03 and LV end-diastolic volume 128.1 [23.5] versus 158.08 [(42.31], P=0.03). In 4-month follow-up, these differences were no longer observed, and both the LV volumes and LVEF were comparable. The comparison of absolute changes of LV volumes showed that there was a significant decrease in ESV and end-diastolic volume in the active treatment group (Table 4). The differences between the groups concerning absolute change of LV volumes were statistically significant for the cell treatment (Figure 3). The results of MRI are consistent with SPECT results on LV function and volumes (data not shown).

There was no significant correlation between the number of injected cells and SDS, SDS%, LVEF, and CCS class. However, the small number of patients precludes the definitive analysis of dose–response.

Clinical Follow-Up

Clinical follow-up was performed after 1 and 4, 6 and 12 months in an outpatient setting. Changes in angina class between baseline and follow-up were evaluated, and comparison of the numbers of patients who had improvement by at least 1 CCS class versus patients who remained in

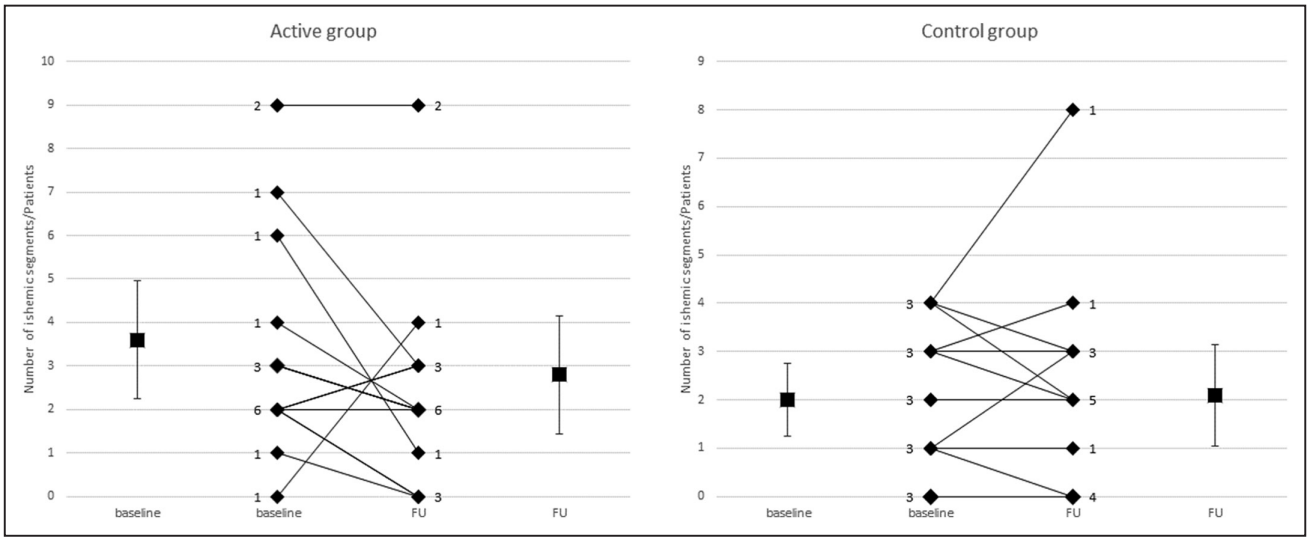

Figure 2. Changes in the extent of inducible ischemia in SPECT (single-photon emission computed tomography) between baseline and 4-month follow-up. The numbers on *y* axis correspond to segments with inducible ischemia. Numbers at the bars denote the number of patients with given change of ischemic segments.

baseline symptoms class was performed. Percentage of patients showing improvement of at least 1 CCS class after 1 month showed no significant differences between the placebo and active treatment arm groups (41.7% versus 58.3%; *P*=0.68). Similarly, there were no significant differences at 4 months (50% versus 33.3%; *P*=0.63), 6 months (70% versus 50.0%; *P*=0.42), and 1 year (55.6% versus 81.8%; *P*=0.33). Additionally, there was no difference in the number of patients with improvement between 4 and 12 months (*P*=0.22). At 1-year follow-up, the doses of nitrates were lower in the cell therapy group (mean 47.5 versus 77.5 mg; *P*=0.058); however, the difference did not reach statistical significance.

Safety Aspects

All serious adverse events were reported to the CRO and adjudicated. There were no deaths and no cases of MI during follow-up. Two serious adverse events were noted in placebo-treated arm (one case of unstable angina [troponin negative] treated medically and one case of deep vein thrombosis) and 3 in the active arm (acute back pain in the lumbar area radiating to abdomen during the mapping procedure, which was related to previously diagnosed spondyloarthritis, that fully resolved after analgesic treatment, one pseudoaneurysm of the femoral artery, and one case of hospital admission for unstable angina [troponin negative], treated medically). At 4- and 12-month follow-up, no evidence of ventricular or supraventricular arrhythmia in the 24-hour ECG monitoring was noted. Laboratory tests after 4 months revealed no significant alterations in comparison to baseline, in particular, no major changes in blood cell counts and plasma CRP (C-reactive protein) levels (Online Table III).

Discussion

The REGENT-VSEL trial investigated the effects of transendocardial delivery of autologous BM-derived CD133+ cells on the myocardial ischemia and angina symptoms in patients with refractory angina. To the best of our knowledge, this is the first placebo-controlled trial using selected CD133+ cells in refractory angina. Study results need to be interpreted with caution because of the lack of statistical power related to smaller than planned number of enrolled patients. The primary finding of the study is that we did not observe benefits of cell therapy concerning the primary and secondary end points. We did not find a significant improvement in the number of ischemic segments in SPECT imaging with adenosine in 4-months follow-up. Also, the LVEF did not change significantly over time. Cell therapy was associated with reduced LV volumes when compared with the placebo, but given the small sample size and normal LV function at baseline, this should be interpreted with caution. Consistently with the SPECT imaging results, the clinical evaluation did not show a significant reduction of the angina CCS class and nitrate use after 1, 4, 6, and 12 months. In the cell treatment arm, the absolute difference of SDS between baseline and follow-up was numerically larger than in placebo group, so it is possible that enrollment of more patients would have led

Table 3. Logistic Regression Model to Identify the Factors Associated With an Improvement of Inducible Ischemia by SPECT

Variable	OR per	OR (95% CI)	Univariate *P* Value
Number of segments with inducible ischemia	1	1.18 (0.86–1.70)	0.29
History of myocardial infarction	Yes/no	1.50 (0.30–8.66)	0.62
Treatment allocation	Active/placebo	3.54 (0.82–17.58)	0.09
Baseline EF (MRI)	1	0.93 (0.83–1.03)	0.17
Baseline EF (SPECT)	1	0.96 (0.88–1.03)	0.26
Baseline EF (MRI, SPECT)	1	0.97 (0.89–1.05)	0.45

CI indicates confidence interval; EF, ejection fraction; MRI, magnetic resonance imaging; OR, odds ratio ; and SPECT, single-photon emission computed tomography.

Table 4. Changes of Left Ventricle Function and Volumes in 4-Month Follow-Up in Cardiac Magnetic Resonance Imaging

	Placebo Group	Stem Cell Group	*P* Value
Left ventricular ejection fraction, %			
Baseline	53.6 (6.6)	48.5 (9.8)	0.13
4-month follow-up	50.6 (7.7)	46.8 (11.0)	0.34
Change from baseline	−2.6 (4.1)	−0.4 (4.3)	0.22
Left ventricular end-systolic volume, mL			
Baseline	59.9 (15.2)	83.4 (32.7)	0.03
4-month follow-up	69.8 (22.1)	84.4 (32.1)	0.18
Change from baseline	7.4 (11.8)	−4.3 (11.3)	0.02
Left ventricular end-diastolic volume, mL			
Baseline	128.1 (23.5)	158.1 (42.3)	0.03
4-month follow-up	139.3 (30.7)	154.9 (35.0)	0.24
Change from baseline	7.4 (15.8)	−9.1 (14.9)	0.02

Data are mean (SD) unless otherwise stated.

to significant results. In particular, given the fact that patients in the active arm had larger SDS numerically at baseline, and the number of responders was higher in that group, the trial provided a signal of improvement. Because the trial is underpowered, it is, however, a hypothesis-generating statement. The results differed from previously published trials, with refractory angina patients treated by transendocardial delivery of BM and mobilized blood-derived mononuclear cells which showed improvement. On the contrary, nonplacebo controlled PROGENITOR trial (Safety and Efficacy of Transendocardial Injection of Autologous Endothelial Progenitor Cell CD 133 for Therapeutics Angiogenesis) did not show improvement with use of selected CD133$^+$ cells.[20] The differences in the outcome of REGENT-VSEL and previously published studies should be interpreted with consideration of the cell type, patients selection, and evolving standard of care of CAD using current interventional approach and medical therapy.

Cell Selection

Immunomagnetic selection can produce well-characterized cellular fraction enriched for stem and progenitor cells. Such an approach with CD133$^+$ and CXCR4$^+$ cells was used in acute MI. However, the data on the effects of selected versus nonselected BM cells in refractory angina are less well investigated.[21,22] Most of the studies with the transendocardial delivery of BM cells used unselected mononuclear cells isolated by Ficoll-gradient centrifugation. Such a population is heterogeneous and contains stem and progenitor cells, but the majority of the committed populations may have distinct effects on myocardium than isolated CD133$^+$ cells. The majority of placebo-controlled studies with mononuclear cells (MNC) showed a sustained improvement of perfusion and LV function, as well as angina symptoms.[12,15] To date, the transendocardial selected CD133$^+$ cells in refractory angina were used in PROGENITOR study only. They in contrast to REGENT-VSEL trial isolated cells from peripheral blood after mobilization of the BM with G-CSF, and the number of cells was 10× greater than in the current study.[20] The number of injected cells was indeed lower than that in ACT34-CMI study (A Double-blind, Prospective, Randomized, Placebo-Controlled Study to Determine the Tolerability, Efficacy, Safety, and Dose Range of Intramyocardial Injections of G-CSF Mobilized Auto–CD34$^+$ Cells for Reduction of Angina Episodes in Patients With Refractory Chronic Myocardial Ischemia), in which patients received either 1×10^5/kg or 5×10^5/kg, which would be ≈20× more than in our study (for a lower dose used in ACT34-CMI study). This is consistent with other studies in which the cells were isolated from mobilized G-CSF blood.[12,15] In the PROGENITOR trial, the target cell dose was 20 to 30×10^6. It might be one of the limitations of an approach of isolating a rare population of cells such as CD133$^+$ from the BM. Studies with BM cells showing positive outcomes used nonselected mononuclear cells; however, for such cells, the yield from BM biopsy is much higher (≤100×10^6 MNC). Second, in PROGENITOR study, the transendocardial injections were performed only in the active treatment arm, while patients from the control group underwent a simulated procedure.[20] In general, our data are similar regarding clinical efficacy to the PROGENITOR study. Both showed no differences between the active treatment and control arms over 4- to 6-month follow-up. There was also no improvement of the objective parameters of ischemia (SPECT with dipyridamole and NOGA mapping) and LV contractility and size in the PROGENITOR trial. Interestingly, they showed improvement of regional contractility evidenced by repeated electroanatomical mapping (an increase of local linear shortening in segments in which injections were performed).[20] One cannot exclude indirect effects of the placebo injections on myocardial perfusion in REGENT-VSEL. Interestingly, in the current study, the absolute change in LV volumes was significant for cell therapy (reduction of LV end diastolic and end systolic volumes). However, the LVEF did not change. There is no data to consider the use of immune-selected cells (either CXCR4 or CD133) as superior to nonselected MNC in stable CAD or acute MI. In fact, majority of clinical trials using CD133 cells provided neutral outcomes, regardless of the route of administration. In the recently published pilot IMPACT-CABG trial, the number of injected cells was ≈10 million; however, there was no benefit of cell implantation over placebo in patients with ICM.[23] Similarly, Cardio133 trial showed no benefits of CD133 cells in 60 patients with stable CAD and impaired LVEF <35% treated by transepicardial injection at the time of CABG in comparison to placebo. There was no improvement of assessed by MRI LV function and remodeling, as well as clinical status and quality of life.[24] These relatively large placebo-controlled studies contradicted the earlier published smaller studies where use of peripheral blood-derived CD133$^+$ cells on top of surgical revascularization or laser revascularization showed promising results.[25,26] In ST-segment–elevation myocardial infarction patients, the use of selected cells also provided a none-to-modest improvement of LVEF in 35 patients with recent MI; however, this group of patients is distinct from REGENT-VSEL population.[21,22] In refractory angina, several trials using nonselected MNC or CD34$^+$ cells showed positive results. The clear difference was the substantially higher number of cells (MNC from the BM or CD34$^+$

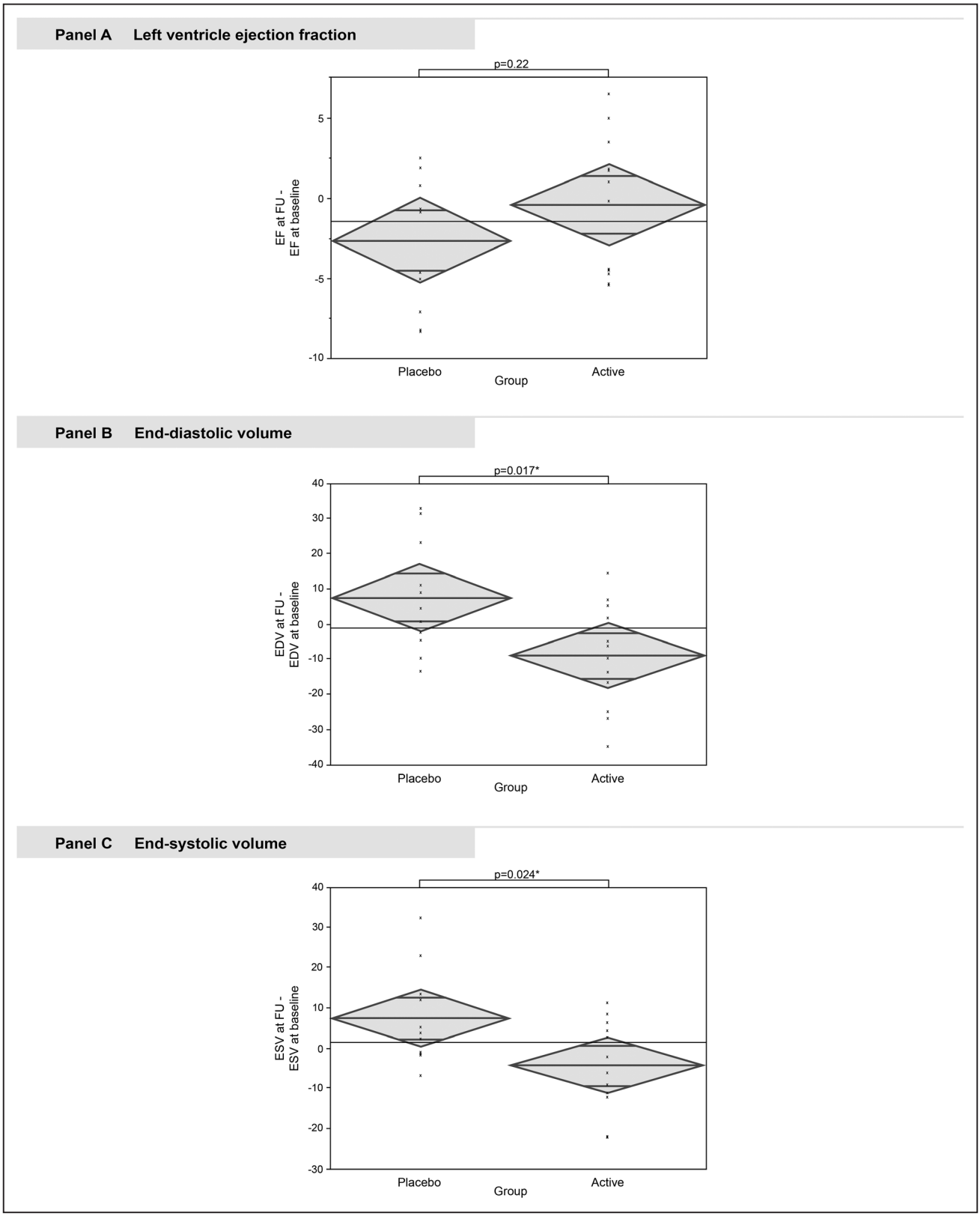

Figure 3. Changes in the left ventricle (LV) ejection fraction (A) and LV volumes (B and C) in magnetic resonance imaging (MRI) between baseline and 4-month follow-up.

from mobilized peripheral blood) used in these studies.[27,28] There are several factors that might be responsible for this: (1) immunoselection leads to relatively small number of cells available for delivery; (2) use of one marker leads to depletion of other marker-negative cells with therapeutic potential (eg, MSC); (3) CD133 is expressed on several types of cells that

do not have therapeutic potential. In general, the available data suggest that selected CD133+ cells are not the optimal cells for treatment of chronic myocardial ischemia.

Patient Selection

Another important point is that given the substantial progress in interventional techniques, such as chronic total occlusion interventions, the number of patients rejected from standard treatment is declining. As an example in the PROGENITOR study, it took 4 years to enroll 28 out of 237 screened patients in 3 experienced high-volume centers. We screened 90 patients over 3 years to enroll 31 patients. It suggests that such therapy applies to a minority of patients with low quality of life and not amenable to revascularization. It is crucial to identify the patients who are responders to cell therapy. Analysis of the individual patient data from SPECT showed that in patients with higher number of segments with abnormal perfusion at baseline, the improvement tends to be more significant. Also, the number of responders in the active treatment arm was significantly higher than those in the placebo group. Logistic regression analysis showed that the history of MI increased the chance of positive response to therapy 1.5× and allocation to the active arm 3.5×. Hypothetically, the inclusion of patients who had a low number of ischemic segments (or small diffusely distributed areas) might have influenced the results because these patients are less likely to benefit from such therapy. The study was designed and approved in 2010 when the percentage of successful recanalizations of chronic total occlusions was much lower than the current one. In many cases, they are highly symptomatic, but because of the anatomy of small vessel disease, they have several smaller areas of reversible perfusion deficit rather than one large one. It may be an additional factor that influenced the outcome of the study.

The ACT34-CMI study showed a sustained benefit regarding angina as well as exercise time after 12 and 24 months after IM administration of CD34+ cells. This trial included patients with higher angina class (III-IV) than ours, which perhaps reflected higher ischemic burden and better potential of cell therapy.[29] Also, there is a reduction of emergency room visits 2 years after the cell therapy, suggesting the long-term efficacy of MNC.[14]

In our study, the placebo group included a numerically higher number of patients with diabetes mellitus (53% versus 25%; *P*=ns) than the placebo group. Such a difference could have been related to the fact that these patients more often do not experience symptomatic improvement after use of nitrates, have atypical symptoms, and experience their side effects. The protocol did not mandate the use of nitrates as an inclusion criterion.

Imaging Modalities

It has been well recognized that stress SPECT may underestimate the extent of CAD compared with coronary angiographic findings, particularly with vasodilator imaging. In the study by Lima et al,[30] only 25% of patients with angiographic 3-vessel CAD had perfusion defects or regional dysfunction in the supply regions of the 3 stenosed coronary arteries. Also with multivessel CAD, the normal region on SPECT often changes in location from rest to stress, potentially masking the extent and severity of multivessel ischemia. We know, however, that renormalization of the images to match their resting level before image interpretation allows diagnosis of contralateral ischemia and significantly improves the detection of multivessel CAD, without a substantive loss in specificity. Also in the present study, a significant coronary stenosis seen on angiography correlated with the myocardial zone of hypoperfusion on SPECT. We should emphasize, however, that many of the patients had rather diffuse a distal disease in 1 to 2 vessels, so they were not typical patients with 3-vessel disease.[30,31] This problem of relation of the SPECT-based findings to cell injection is difficult for several reasons: (1) variability of SPECT results, especially when the baseline area of perfusion of deficit is small; (2) although anatomically the areas of reversible perfusion defects were consistent with the areas of hibernation in NOGA, which were places of cells injection we cannot be confident that injections were done in precisely the same areas as shown in SPECT. Also, some patients had 2 to 3 distinct areas of hibernation, and in some cases, because of technical challenges, it was not possible to inject cells to all target zones (eg, because of papillary muscle, chordae). Study of van Ramshorst et al provided one of the largest cohorts of patients treated with NOGA and showed that among predictors of the responder status were diabetes mellitus, the number of perfusion deficits, and higher the summed stress score (larger the ischemic zone, the better the outcome).[32]

Importantly, data from >100 patients showed that finally, only ≈50% of injected segments had improved perfusion as evaluated by SPECT.[32] Many of patients in our study had a moderate extent of perfusion deficit, and it could have impacted the results.

The MRI imaging was in principle used to detect a potential decrease of LVEF related to recurrent ischemia during the follow-up and to document the increase of LVEF after cell therapy. Also, van Ramshorst et al in a relatively large cohort of patients with refractory angina showed an increase of LVEF after 3 months post-cell delivery in a population similar to ours. Their finding was an increase of LVEF related to decreased LVESV.[32] Also, the role of LVEF as a surrogate end point for cell therapy studies needs to be established.

Safety

The safety of transendocardial procedures was excellent with no major complications and life-threatening adverse events. We did not report any case of cardiac tamponade or pericardial effusion and only one pseudoaneurysm of the femoral artery. There were no deaths and MIs, as well as arrhythmic events throughout the follow-up. The key factor is a measurement of myocardial thickness and avoidance of injections in the thickened wall in the region of the post-infarct scar. One potential problem with this technology is peripheral artery disease frequent in this population of patients. Because the access sheath is 8F, in cases of severe iliac and aortic tortuosity, we used long sheaths to navigate with the electrode. Also in 2 cases with calcified cusps of the aortic valve, we used the long 8F shuttle sheath over the wire placed in the LV to avoid the difficult passage of electrodes through the valve.

Limitations

Because of slow recruitment at the single center, the study was stopped before reaching the planned number of randomized

patients, which significantly reduced the statistical power to detect the potential efficacy of cell therapy. The double-blinded randomized design with placebo-control and independent assessment of the results might have reduced this limitation to some extent. Importantly, no patients were lost to follow-up and primary end point data available for all patients. Also, the results are consistent across the imaging and clinical end points and comparable to other studies with selected CD133+ cells. Nevertheless, the study is underpowered, and the results would be interpreted with caution. Other factors that might have affected the results is the relatively low number of injected cells and shortcomings of SPECT as a tool for evaluating this group of patients. The retrospective power calculation suggests that 250 to 330 patients would be needed to show the difference in the primary end point. The small number of patients could also have an influence on the interpretation of the logistic regression results, so they should be regarded a hypothesis generating. Regarding secondary end point, we were not able to obtain paired MRI images from 5 patients. To increase the validity of the analyses, the SPECT and MRI images were reviewed by 2 experienced imaging specialists blinded to the treatment assignments. Also, the study investigators remained blinded as to the allocation of the individual patients until final 1-year clinical follow-up, with the quality of life assessment is completed. The median number of injections was 10, but not all patients in active treatment arm received the same number of cells, so it could have some influence on the outcomes.

Conclusion

In conclusion, because of lack of statistical power, the study cannot conclusively validate the efficacy of CD133+ cells in the treatment of nonoption patients with refractory angina. It confirmed the safety of transendocardial cell delivery and highlight the limitations associated with this particular protocol of cell isolation.

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Disclosures

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