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Long-term clinical outcome after intracoronary application of bone marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival

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Background In the REPAIR-AMI trial, intracoronary infusion of bone marrow-derived cells (BMCs) was associated with a significantly greater recovery of contractile function in patients with acute myocardial infarction (AMI) at 4-month follow-up than placebo infusion. The current analysis investigates clinical outcome and predictors of event-free survival at 5 years.

Methods and results In the multicentre, placebo-controlled, double-blind REPAIR-AMI trial, 204 patients received intracoronary infusion of BMCs ($n = 101$) or placebo ($n = 103$) into the infarct vessel 3–7 days following successful percutaneous coronary intervention. Fifteen patients died in the placebo group compared with seven patients in the BMC group ($P = 0.08$). Nine placebo-treated patients and five BMC-treated patients required rehospitalization for chronic heart failure ($P = 0.23$). The combined endpoint cardiac/cardiovascular/unknown death or rehospitalisation for heart failure was more frequent in the placebo compared with the BMC group (18 vs. 10 events; $P = 0.10$). Univariate predictors of adverse outcomes were age, the CADILLAC risk score, aldosterone antagonist and diuretic treatment, changes in left ventricular ejection fraction, left ventricular end-systolic volume, and N-terminal pro-Brain Natriuretic Peptide (all $P < 0.01$) at 4 months in the entire cohort and in the placebo group. In contrast, in the BMC group, only the basal ($P = 0.02$) and the stromal cell-derived factor-1-induced ($P = 0.05$) migratory capacity of the administered BMC were associated with improved clinical outcome.

Conclusion In patients of the REPAIR-AMI trial, established clinical parameters are associated with adverse outcome at 5 years exclusively in the placebo group, whereas the migratory capacity of the administered BMC determines event-free survival in the BMC-treated patients. These data disclose a potency–effect relationship between cell therapy and long-term outcome in patients with AMI.

Keywords Acute myocardial infarction • Bone marrow-derived cells • Cell therapy • Clinical outcome

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Introduction

Adverse remodelling and subsequent development of post-infarction heart failure determine long-term clinical outcome after acute myocardial infarction (AMI).[1,2] Based on a number of experimental studies demonstrating that administration of bone marrow-derived mononuclear cells (BMCs) beneficially interferes with adverse remodelling following the induction of AMI,[3] numerous clinical trials have been initiated to investigate the effects of intracoronary application of BMC in patients with AMI. A recent meta-analysis of the published trials revealed modest, but significant benefits on parameters of left ventricular (LV) remodelling,[4] while the individual results of these trials were heterogeneous with respect to surrogate endpoints like ejection fraction (EF) or LV volume changes 4–6 months after BMC administration. The largest meta-analysis so far including >2600 patients even suggested that BMC administration may reduce the incidence of death and major adverse cardiac events during longer term follow-up.[5] However, inherent to all meta-analyses, the degree of heterogeneity among the trials included as well as a potential publication bias in favour of trials with a positive outcome constitute a major limitation of such analyses.

Here, we report the 5-year clinical follow-up results of the patients treated with intracoronary administration of BMC or placebo within the so far largest placebo-controlled, randomized trial, the REPAIR-AMI trial. Specifically, we sought to identify independent predictors of clinical outcome after AMI and how intracoronary administration of BMC might interact with these independent predictors, in order to potentially establish a potency–effect relationship between BMC administration and long-term clinical outcome.

Methods

Study population and protocol

The study protocol has been described in detail previously.[6–8] In brief, patients aged 18–80 years were eligible for inclusion into the study, if they had an acute ST elevation myocardial infarction successfully reperfused with stent implantation with a residual significant LV regional wall motion abnormality. The ethics review board of each individual participating centre approved the protocol, and the study was conducted in accordance with the Declaration of Helsinki. The study is registered with clinicalTrials.gov (number NCT00279175).

In this double-blind, placebo-controlled, randomized trial, performed in 17 centres, at a median of 4 days after AMI reperfusion therapy, bone marrow aspiration was performed in 204 patients, and the aspirate was sent to a central cell processing laboratory (Institute for Transfusion Medicine, Frankfurt, Germany), where patients were randomized to placebo medium or BMC receiving groups. BMC or placebo was infused using the stop-flow technique via an over-the-wire balloon and positioned in the infarct-related coronary artery within the segment of the previously implanted stent.

The results for the primary endpoint, change of LVEF by LV angiography assessed at 4 months, as well as the 12- and 24-month clinical outcome have been previously reported.[6,7,9] At baseline, in five patients, no LV angiography data were available for the following reasons: two patients did not receive study therapy, one patient had an LV thrombus (all three patients in the placebo group), one LV angiography could not be analysed due to insufficient contrast opacification, and one angiography was not performed for unknown reasons (both patients in the BMC group). At follow-up, in the BMC group, two patients died, two declined, one had poor-quality results on angiography, and one patient did not undergo angiography, whereas in the placebo group, two patients died, three declined, one had poor-quality results, and three patients did not undergo angiography. Thus, paired LV angiography data were available in 92 placebo and 95 BMC-treated patients. For analysis of the primary endpoint, the study analysing centre had been unblinded after all 4-month data had been collected and finally analysed. However, patients, study centres, investigators, and those entering the data into the database still remained blinded until 12-month follow-up had been completed, and clinical events had been finally categorized. Thereafter, patients and investigators were unblinded. However, database entry and categorization of events were performed unaware of the randomization status by an independent data manager and event committee, respectively.

Endpoints

Five-year clinical event analyses were performed according to a study protocol amendment filed on 30 May 2006. The following events were assessed, as described previously in detail:[7] death of any cause and type of death (cardiac, cardiovascular, or non-cardiovascular), recurrent myocardial infarction (MI), revascularization procedures (percutaneous coronary intervention or coronary artery bypass grafting), stent thrombosis, syncope, ventricular arrhythmias, stroke, or cancer. Rehospitalization due to heart failure was defined as hospitalization with typical clinical findings of heart failure requiring the addition of medication for the treatment of heart failure.

Pre-specified combined clinical endpoints were death, repeated MI or any revascularization procedure, an endpoint reflecting progression of vascular disease, as well as death, MI, or rehospitalization for heart failure, reflecting progression of disease towards heart failure.

It is important to state that the sample size of the REPAIR-AMI trial was not powered to definitely answer the question whether BMC administration is capable to modify mortality and morbidity after AMI.

Assessment of BMC characteristics

Bone marrow-derived cells (BMCs) were obtained as previously described.[6] After Ficoll density gradient centrifugation, BMC subpopulations were quantified by fluorescence-activated cell sorting($CD34^+/CD45^+$, $CD133^+/CD45^+$, and $CD45^+/KDR^+$ cells). Gating was performed according to the international society of hematotherapy and graft engineering guideline.[10] The migratory capacity of isolated BMC was assessed in a modified Boyden chamber assay.[11] In brief, a total of 1×10^6 BMCs were resuspended in 250 μL X-*vivo* 10 medium and placed in the upper chamber of a modified Boyden chamber filled with Matrigel (BioCoat invasion assay, 8 μm pore size, Becton Dickinson). Then, the chamber was placed in a 24-well culture dish containing 500 μL of endothelial basal medium medium. For stimulation of invasion, 100 ng/mL of stromal cell-derived factor-1 (SDF-1) was added in the lower chamber. After incubation for 24 h at 37°C, transmigrated cells were counted by independent investigators. Invasion assays were run in duplicates. Migration was assessed under basal conditions and after stimulation with SDF-1.

CADILLAC risk score

The CADILLAC risk score was calculated as previously described.[12] For age >65 years, two points were assigned, for Killip class 2–3 three points, baseline LVEF <40% four points, anaemia two points, renal insufficiency three points, triple-vessel disease two points, and for post-procedural thrombolysis in myocardial infarction flow grade 0–2 two points.

Statistics

All data were analysed according to the intention-to-treat design. Continuous variables are presented as mean ± SD (if not stated otherwise).

Categorical variables shown as median with 25 / 75 percentile, and were compared with the χ^2 test or Fisher's exact test. Continuous variables were compared using ANOVA.

Time-dependent event rates were estimated by Kaplan–Meier survival curves for the randomization status, and *P*-values were determined by use of log-rank statistics. The exact event date was used for defining time to event for single events such as death. Otherwise, the event date of the first event that occurred in a patient was used in the case of combined endpoints. Unadjusted Cox regression analysis was used to assess the hazard ratios (HRs) of the randomization status related to the clinical endpoint to be assessed. Owing to the limited number of patients, no statistical comparison was performed between the groups BMC and placebo for single events except total and cardiac mortality. In addition, Wei–Lin–Weissfeld analyses were performed to account for the limited number of events and to increase the statistical power.[13] Statistical significance was assumed if $P < 0.05$. Reported *P*-values are two-sided. Statistical analyses were performed using SPSS (Version 20, SPSS, Inc.).

Results

Patient population and baseline characteristics

A total of 204 patients were randomized: 103 received intracoronary administration of placebo and 101 received intracoronary administration of BMC. No significant differences in baseline characteristics were present (see Supplementary material online, *Table S1*).[6,7] Pharmacological treatment did not significantly differ between placebo and BMC at 5-year follow-up, although the use of aldosterone antagonists was significantly less frequent in the BMC group at hospital discharge and at 12-month follow-up (see Supplementary material online, *Table S2*).

Clinical events at 5-year follow-up

As illustrated in the study flowchart (*Figure 1*), 5-year follow-up of clinical events was completed in 98% of the patients. Mean follow-up time was 58 ± 15 (median 61) months. In three patients, only vital status could be obtained by the registration office, but no further information was available. Thus, these patients were only counted for mortality analyses.

A total of 22 deaths occurred during 5-year follow-up (*Table 1*). The corresponding Kaplan–Maier survival curve is shown in *Figure 2A*. Mode-of-death analysis revealed that cardiac death occurred in eight placebo- compared with four BMC-treated patients. Interestingly, death attributed to progression of heart failure occurred in three patients of the placebo group, but in none of the patients in the BMC group. In contrast, sudden cardiac death was equally distributed. In addition, one patient in each group died from stroke, and three placebo- and two BMC-treated patients died from other causes.

Table 2 summarizes the other pre-specified clinical endpoints. There were no differences with respect to ischaemic or arrhythmic events, or rehospitalization for heart failure. New cases of cancer were diagnosed in seven placebo- and four BMC-treated patients.

The pre-specified combined endpoint death, recurrence of MI, or any revascularization was significantly reduced in the BMC group

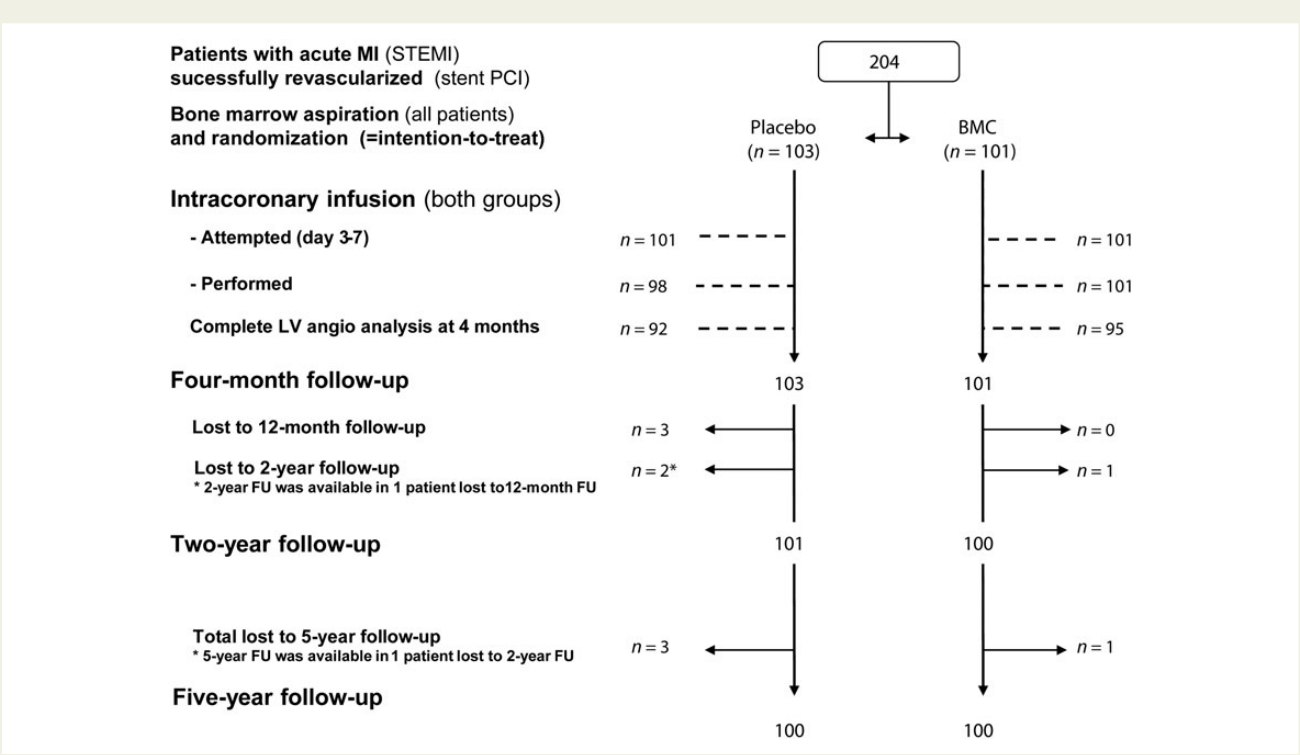

Figure 1 Trial flowchart of the REPAIR-AMI trial.

Table 1 Death and mode of death

Per patient analysis	Placebo ($n = 100$)	BMC ($n = 100$)
Death (*n*)	15	7
Cardiac (*n*) (AMI, SCD, CHF, myocardial rupture)	8	4
SCD	3	3
CHF-related death	3	0
Cardiovascular (*n*) (stroke)	1	1
Non-cardiovascular (*n*)	3	2
Cause of death unknown[a]	3	0

[a]Information of registration office, patients only included into mortality analyses.

compared with the placebo group (*Figure 2B*). Likewise, the combined endpoint death and rehospitalization for heart failure were reduced, albeit not significantly, in the BMC-treated patients compared with the placebo group (*Figure 2C*). These findings are corroborated by the illustration of the HRs, demonstrating that all combined endpoints favour the BMC treatment group (*Figure 3*). Analysis of repeated cardiovascular events according to the Wei–Lin–Weissfeld model revealed that the cumulative endpoint death and rehospitalisation for heart failure [HR 0.48, 95% confidence interval (CI) 0.25, 0.93] as well as death and rehospitalization for heart failure and MI (HR 0.52, 95% CI 0.31, 0.87) were significantly reduced. In contrast, taking into account repeated revascularization procedures, the prespecified combined endpoint death, MI, and revascularization were no longer significantly different between the BMC- and the placebo-treated patients (HR 0.77, 95% CI 0.55, 1.08; see Supplementary material online, *Table S3*).

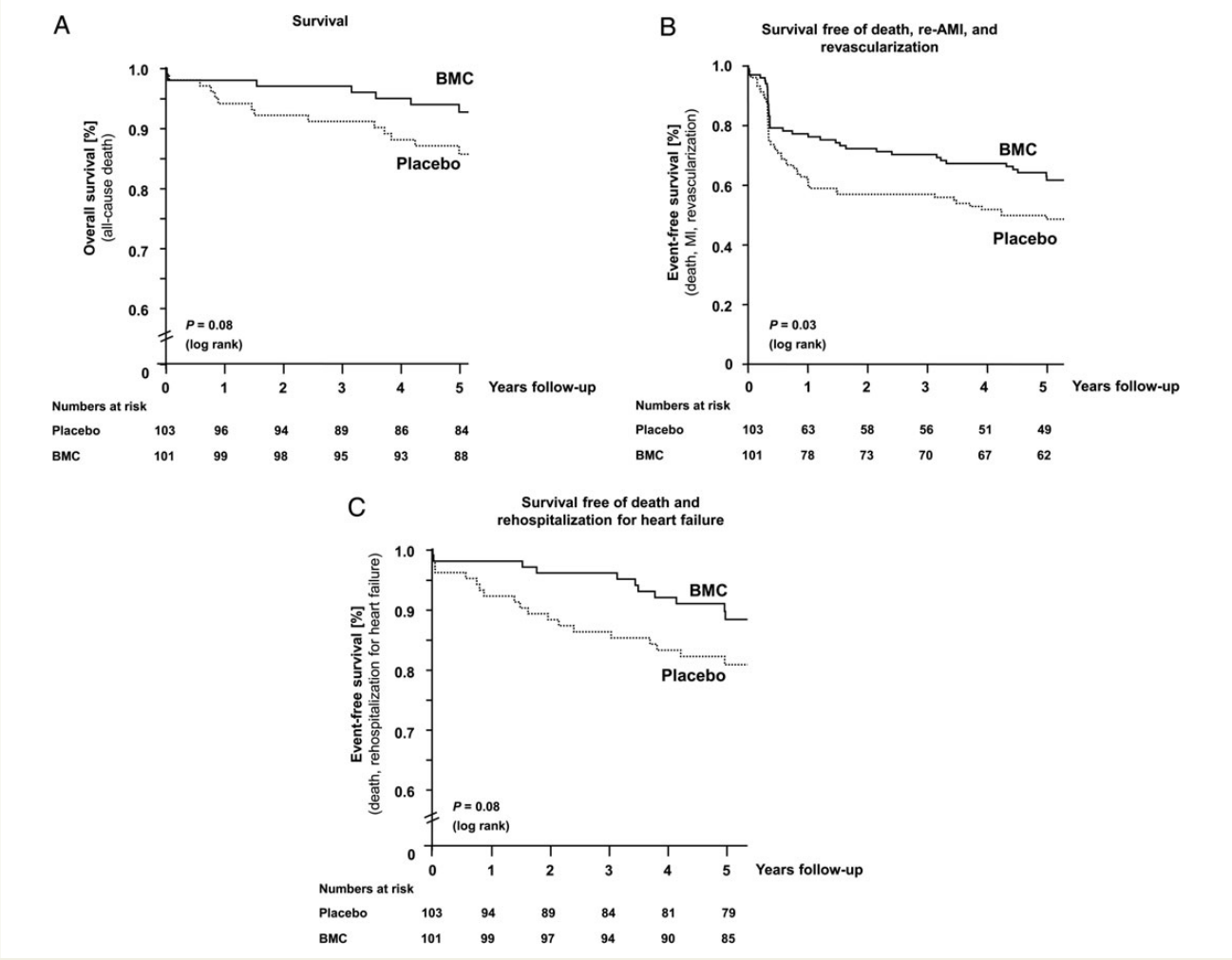

Figure 2 (*A*) Kaplan–Meier analysis for total mortality in the placebo- and the BMC-treated patients at 5-year follow-up. Comparison between groups is performed using the log-rank test. (*B*) Kaplan–Meier analysis for survival free of death, recurrent myocardial infarction, and revascularization in the placebo- and the bone marrow-derived cell-treated patients at 5-year follow-up. Comparison between groups is performed using the log-rank test. (*C*) Kaplan–Meier analysis for survival free of death and rehospitalization for heart failure in the placebo- and the bone marrow-derived cell-treated patients at 5-year follow-up. Comparison between groups is performed using the log-rank test.

Table 2 Clinical endpoints

Per patient analysis	Placebo ($n = 99$)	BMC ($n = 98$)
Myocardial re-infarction (STEMI/NSTEMI) (*n*)	7	5
Acute coronary syndrome (unstable angina) (*n*)	10	8
Rehospitalization for heart failure (*n*)	9	5
Revascularization (i)	42	30
Target vessel revascularization (*n*)	28	18
Non-target vessel revascularization (*n*)	18	14
Stroke (*n*)	7	3
Ventricular arrhythmia or syncope (*n*)	7	6
Pacemaker implantation (*n*)	1	1
ICD implantation (*n*)	10	4
Primary prevention (*n*)*	9	2
Secondary prevention (*n*)	1	2
New CRT therapy (*n*)	2	1
Cancer (*n*)	7	4

CRT, cardiac resynchronization therapy; ICD, internal cardioverter defibrillator; NSTEMI, Non-ST elevation myocardial infarction; STEMI, ST elevation myocardial infarction.
*$P < 0.05$.

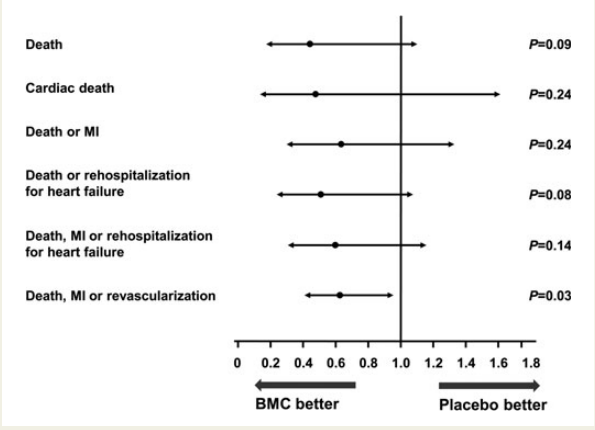

Figure 3 Hazard ratios of intracoronary bone marrow-derived cell administration with respect to individual and combined clinical events at 5-year follow-up (Cox regression analysis).

Predictors of cardiovascular death and rehospitalization for heart failure

To identify individual predictors of adverse cardiovascular outcome and the potential interaction with BMC administration, we combined cardiac and cardiovascular death, death of unknown origin and rehospitalization for heart failure, the major goal of regenerative therapeutic attempts. A total of 28 patients suffered from these events, 18 in the placebo group and 10 in the BMC group (P log-rank = 0.10) during 5-year follow-up. As illustrated in *Table 3*, in the total study population, patients suffering from an event were significantly older, had more frequent hypertension and multi-vessel coronary artery disease, a higher CADILLAC risk score, and received more heart failure directed pharmacological treatment (aldosterone antagonists and diuretics) at baseline. With respect to quantitative parameters of LV function, these patients had significantly lower LVEF, increased LV end-systolic volumes, and higher N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) serum levels at 4 months, while these parameters did not differ at baseline (data not shown). Accordingly, a decrease in LVEF as well as an increase in LV end-systolic volumes and NT-proBNP levels from baseline to 4 months were the strongest individual predictors of a worse clinical outcome, thus documenting the crucial role of early (4 months) adverse LV remodelling to be associated with adverse long-term clinical outcome.

Analysing the placebo and the BMC group separately revealed that all parameters of adverse LV remodelling at 4 months were fully retained as individual predictors of adverse long-term clinical outcome in the placebo group, but not in the BMC group (*Table 3*). Most importantly, analysing the number, composition, and migratory capacity of the cellular product administered in the BMC group revealed that patients suffering from an event during follow-up received BMC with a profoundly reduced migratory capacity, while the total number of administered BMC as well as the frequency of specific progenitor cell subpopulations such as CD34- or CD133-positive cells did not differ (*Table 4*). This result was confirmed by Kaplan–Meier analysis of the BMC-treated patients, demonstrating that patients receiving BMC with a high-migratory capacity had a significantly better ($P = 0.01$) event-free survival (*Figure 4*). The lack of any association between clinical outcome and BMC characteristics in the placebo group, which underwent BMC aspiration and cell isolation, but did not receive intracoronary administration of the cells, excludes the possibility that the impaired migratory capacity of the autologous BMC simply reflects a patient-inherent marker of increased risk, irrespective of intracoronary administration of the isolated BMC. Taken together, these data point towards a 'potency–effect' relationship, whereby the migration capacity of the administered BMC determines long-term clinical outcome by ameliorating adverse LV remodelling early (within 4 months) after an AMI.

Discussion

The results of the so far largest placebo-controlled multicentre REPAIR-AMI trial document the long-term safety of intracoronary administration of BMC in patients with AMI. Although the study was not powered to definitively show an effect of BMC administration on clinical outcome, the present 5-year clinical follow-up analysis demonstrated that all individual major adverse clinical endpoints tended to occur less frequently in the BMC group, with some of the combined endpoints even approaching statistical significance in this trial with 204 AMI patients. Most importantly, however, our results for the first time disclose an association between the migratory capacity of the administered BMC, and long-term clinical outcome, thus suggesting the presence of a 'potency–effect' relationship.

In line with previous studies,[14–17] the present results document that adverse LV remodelling, as evidenced by a decrease in LVEF, an increase in LV end-systolic volume, and persistently elevated

Table 3 Significant predictors of adverse clinical outcome (cardiac, cardiovascular and unknown death, and rehospitalization for heart failure) analysed for the total cohort and separately for the placebo and the bone marrow-derived cell groups

	Total cohort			Placebo			BMC		
	No event ($n = 176$)	Event ($n = 28$)	*P*-value	No event ($n = 85$)	Event ($n = 18$)	*P*-value	No event ($n = 91$)	Event ($n = 10$)	*P*-value
Age (years)	55 ± 11	61 ± 10	**0.01**	56 ± 11	64 ± 10	**0.005**	55 ± 12	57 ± 10	0.63
Hypertension (%)	54	79	**0.02**	57	78	0.09	52	80	0.09
Hypercholesterolaemia (%)	57	46	**0.28**	64	39	**0.05**	52	60	0.62
Extent of CAD: 1/2/3 vessel disease (%)	63/26/11	36/39/25	**0.02**	62/28/10	39/44/17	0.18	64/23/13	30/30/40	0.05
CADILLAC score	2; 0/4	4; 2/6	**<0.001**	2; 0/4	4; 2/6	**0.001**	2; 0/4	4; 2/7	0.08
Baseline aldosterone blocker (%)	7	31	**<0.001**	11	41	**0.002**	4	11	0.38
Baseline diuretics (%)	35	58	**0.03**	33	59	**0.04**	37	56	0.29
Baseline LVEF (%) (*n*)	48 ± 10 (174)	43 ± 13 (25)	**0.017**	47 ± 10 (84)	44 ± 12 (16)	0.24	48 ± 9 (90)	40 ± 15 (9)	0.02
Four months LVEF (%); (*n*)	53 ± 11 (169)	42 ± 16 (19)	**<0.001**	52 ± 11 (79)	37 ± 16 (13)	**<0.001**	54 ± 10 (90)	51 ± 11 (6)	0.55
Abs. delta LVEF (%); (*n*)	5 ± 7 (169)	−3 ± 8 (19)	**<0.001**	4 ± 5 (79)	−5 ± 8 (13)	**<0.001**	6 ± 7 (90)	3 ± 3 (6)	0.31
Baseline ESV (mL) (*n*)	70 ± 27 (174)	79 ± 42 (25)	0.91	73 ± 29 (84)	80 ± 43 (16)	0.98	68 ± 25 (90)	76 ± 42 (9)	0.65
Four months ESV (mL) (*n*)	71 ± 35 (169)	98 ± 58 (19)	**0.003**	75 ± 39 (79)	112 ± 60 (13)	**0.003**	67 ± 30 (90)	63 ± 39 (6)	0.78
Abs. delta ESV (mL) (*n*)	0.6 ± 20 (169)	19 ± 26 (19)	**<0.001**	2 ± 20 (79)	28 ± 26 (13)	**<0.001**	−0.7 ± 19 (90)	0.8 ± 15 (6)	0.71
Baseline NT-proBNP (pg/mL); (*n*)	1161; 646/2177 (105)	1985; 1017/3241 (13)	0.37	1125; 755/1749 (45)	2394; 981/3414 (8)	0.41	1208; 644/2366 (60)	1985; 1069/2362 (5)	0.81
Four months NT-proBNP (pg/mL); (*n*)	388; 201/629 (103)	1278; 577/2023 (13)	**<0.001**	401; 208/688 (45)	1754; 554/4258 (8)	**0.001**	371; 176/620 (59)	883; 440/1374 (5)	0.24
Abs. delta NT-proBNP (pg/mL); (*n*)	−709; −1338/−247 (103)	−797; −1168/6 (13)	**0.002**	−709; −1305/−295 (45)	−354; −1612/2926 (8)	**0.006**	−676; −1400/−236 (59)	−1062; −1168/−468 (5)	0.75

Values are shown in mean ± SD or median; 25th/75th percentile; as appropriate. Numbers in parenthesis indicate *n*.
CAD, coronary artery disease; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.
Bold values indicate significant values ($P < 0.05$).

Table 4 Cell characteristics as predictors of adverse clinical outcome (cardiac and cardiovascular death and unknown death, and rehospitalization for heart failure) analysed separately for the placebo and the bone marrow-derived cell groups

	Placebo			BMC		*P*-value
	No event ($n = 85$)	Event ($n = 18$)	*P*-value	No event ($n = 91$)	Event ($n = 10$)	
Total mononuclear cells isolated ($\times 10^6$)	210; 111/309	330; 101/561	0.07	198; 128/286	182; 127/268	0.99
Viability (%)	99; 98/99	99; 98/99	0.87	99; 98/99	97; 97/99	**0.001**
Neutrophil lineage cells (%)	38; 32/47	41; 30/47	0.40	36; 27/50	35; 28/52	0.90
Lymphocytes (%)	43; 37/50	40; 33/49	0.37	42; 34/52	42; 38/60	0.48
$CD34^+CD45^+$ (%)	1.4; 1.0/2.0	1.5; 0.9/2.2	0.73	1.3; 0.9/1.9	1.9; 1.3/2.0	0.23
$CD133^+CD45^+$ (%)	1.1; 0.8/1.5	1.3; 0.8/1.5	0.93	1.0; 0.7/1.3	1.4; 0.9/1.6	0.24
$CD45^+KDR^+$ (%)	0.06; 0.03/0.09	0.06; 0.03/0.10	0.81	0.06; 0.03/0.10	0.06; 0.03/0.17	0.53
Basal migration (migrated cells/10^6 cells)	87; 52/126	61; 34/103	0.17	88; 53/147	42; 23/82	**0.02**
SDF-1-induced migration (migrated cells/10^6 cells)	161; 90/211	161; 75/202	0.72	178; 100/230	129; 69/152	**0.05**

Values are shown as median; 25th/75th percentile.
Bold values indicate significant values ($P < 0.05$).

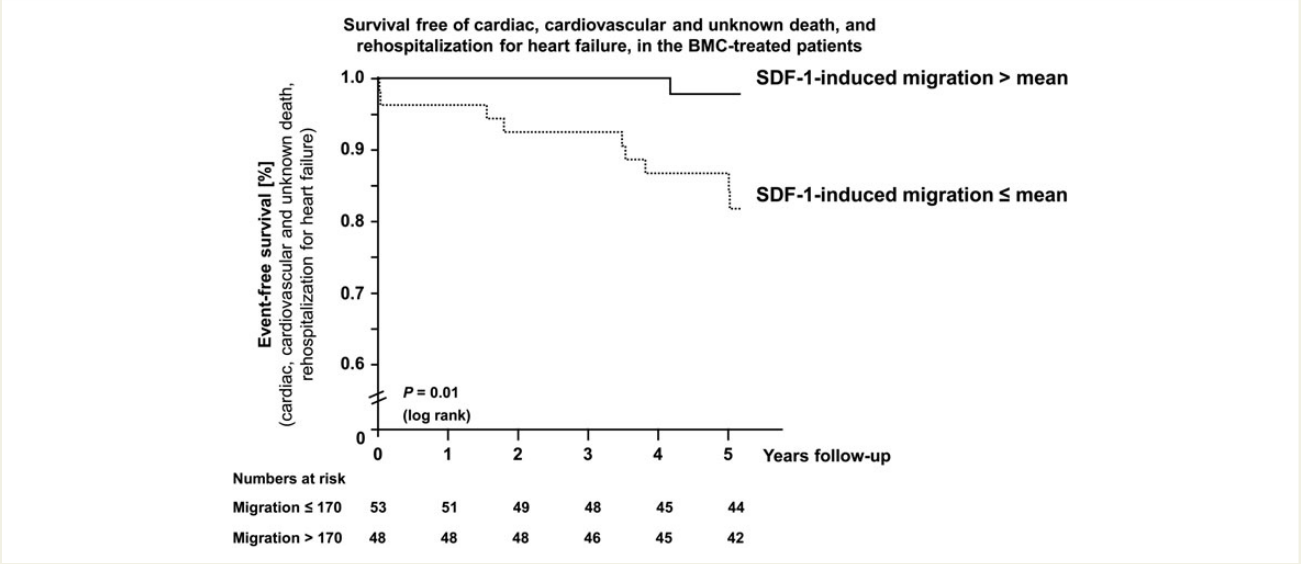

Figure 4 Kaplan–Meier analysis for survival free of cardiac, cardiovascular, unknown death, and rehospitalization for heart failure in the bone marrow-derived cell-treated patients, stratified according to the mean stromal cell-derived factor-1-induced migratory capacity of the administered bone marrow-derived cells, at 5-year follow-up. Comparison between groups is performed using the log-rank test.

NT-proBNP serum levels within 4 months following AMI, is the most important independent predictor of a worse long-term clinical outcome. Thus, therapeutic attempts directed at inhibition or amelioration of LV adverse remodelling following AMI beneficially interfere with long-term clinical outcome, as is well established for angiotensin converting enzyme (ACE) inhibitor therapy[18] and aldosterone blockade.[2]

Cell therapy offers the potential to further ameliorate adverse LV remodelling on top of evidence-based pharmacological treatment. Indeed, although potential mechanisms are still controversially discussed, recent meta-analyses of clinical trials demonstrated that, at least in patients with AMI, intracoronary administration of BMC is associated with a significantly greater recovery of LVEF and a considerable reduction in LV end-systolic volume expansion at 4- to 6-month follow-up.[19,20] The results of the present analysis suggest that the moderate improvement in LVEF of 2.5 absolute percent points in the BMC group compared with the placebo group accompanied by an inhibition of LV end-systolic volume expansion at 4 months, as previously reported,[6] may indeed translate into a long-term clinical benefit at 5-year follow-up. The use of aldosterone antagonists and ACE inhibitors/angiotensin-receptor blockers was slightly, but significantly less frequent in the BMC group at 1 year. Given that these drugs are well

established to improve long-term clinical outcome post-AMI, this disparity might have essentially confounded an even more profound beneficial effect of BMC administration.

The SDF-1/CXCR4 axis is well established to play a crucial role in myocardial repair.[21] Since the original description of SDF-1 to mediate recruitment of BMCs to the sites of vascular and myocardial injury,[22,23] numerous experimental studies have demonstrated the importance of the SDF-1/CXCR4- axis for the recruitment and retention of exogenously administered cells.[24,25] Our preclinical validation studies revealed a close correlation between both basal and SDF-1-induced migratory capacity of BMC and cell retention in ischaemic hindlimb tissue, which was mirrored by similar associations with their neovascularization capacity.[25–28] Moreover, up-regulation of CXCR4 profoundly increased the recruitment of exogenously administered cardiac progenitor cells and improved contractile recovery in a mouse model of AMI.[24] Recently, Wu and co-workers provided convincing experimental and clinical evidence by molecular imaging that early retention of exogenously administered stem cells predicts late cardiac functional recovery implying a 'dose-effect' relationship.[29,30] In our previous clinical studies, we noticed that the SDF-1-induced migratory capacity of intracoronary administered BMC correlated with infarct size reduction as measured by magnetic resonance imaging-derived late enhancement volume in patients with AMI.[31] In addition, in REPAIR-AMI, the SDF-1-induced migratory capacity was significantly and independently associated with improved LVEF recovery at 4 months.[11] However, migratory capacity as measured in our *in vitro* assay may reflect both the number of CXCR4+ cells and their intrinsic migratory capacity, which may be of relevance for future cell enhancement strategies. Taken together, the results of the present study now disclose that the migratory capacity of the administered BMC is associated with clinical outcome after 5 years, thus linking improved early LV contractile recovery with a better long-term clinical outcome to the potency of the administered cells.

Limitations

Some limitations of our study merit further discussion. First, the number of events is rather small. We therefore applied the Wei–Lin–Weissfeld model, which includes repeated cardiovascular events, in order to increase the statistical power. Secondly, there is a slight, albeit statistically non-significant imbalance in the frequency of diabetes between the placebo and BMC groups. Thirdly, none of the statistical interaction tests provided significant results due to the limited number of patients and events in this exploratory *post hoc* analysis. Finally, also due to the limited number of patients included into the study, we did not perform a separate analysis for patients with LVEF of $<45\%$, which is the patient cohort at risk for further cardiovascular events. However, the answer to this question will be investigated in the currently recruiting, EU-funded Phase III outcome study, the BAMI trial (NCT 01569178).

Supplementary material

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

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Conflict of interest: S.D. and A.M.Z. report that they are cofounders of t2cure, a for-profit company focused on regenerative therapies for cardiovascular disease. They serve as scientific advisers and are shareholders.

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