Cell-based therapy for myocardial repair in patients with acute myocardial infarction: Rationale and study design of the SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction (SWISS-AMI)

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Background Recent studies report that intracoronary administration of autologous bone marrow mononucleated cells (BM-MNCs) may improve remodeling of the left ventricle after acute myocardial infarction (AMI). Subgroup analysis suggest that early treatment between days 4 and 7 after AMI is probably most effective; however, the optimal time point of intracoronary cell administration has never been addressed in clinical trials. Furthermore, reliable clinical predictors are lacking for identifying patients who are thought to have most benefit from cellular therapy.

Study Design In a multicenter trial, 192 patients with AMI successfully treated by percutaneous coronary intervention (PCI) of the infarct-related artery will be randomized in a 1:1:1 pattern to 1 control and 2 BM-MNC treatment groups. The control group will be treated with state-of-the-art medical management. The treatment groups will receive intracoronary administration of autologous BM-MNC at 5 to 7 days or 3 to 4 weeks after the initial event, respectively. Left ventricular function as well as scar size, transmural extension, and regional wall motion score will be assessed by cardiac magnetic resonance (CMR) studies at baseline and after 4 and 12 months.

Methods Fifty milliliters of bone marrow will be harvested by aspiration from the iliac crest and then carried by courier to a centralized cell processing facility. The mononucleated cell fraction will be isolated by density gradient centrifugation, washed, and resuspended in 10 mL of injection medium. The cells will be characterized by fluorescence-activated cell sorting analysis and tested for sterility and potency both "in vitro" and "in vivo." Bone marrow MNC will then be reinfused directly in the infarct-related coronary artery.

End points The primary end point is the change in global left ventricular (LV) ejection fraction by CMR at 4 months as compared to baseline. Comparisons will then be made between each of the prespecified therapy subgroups (early and late after AMI) and the control group. Secondary end points include change in infarct size, change in regional myocardial thickness, and wall motion at 4 and 12 months compared to baseline. Infarct extension (size and transmural extension), time delay to PCI, and coronary flow characteristics after PCI will be assessed as potential predictors of LV remodeling and change after cell therapy. Major adverse cardiac events (MACE) (death, myocardial infarction, coronary revascularization, rehospitalization for heart failure) will be assessed at 4, 12, and 24 months and time to MACE will be estimated.

Discussion With the present study, we aim to determine the optimal time point of intracoronary administration of autologous BM-MNC after AMI on LV remodeling. (Am Heart J 2010;160:58-64.)

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Table I. Randomized clinical trials of intracoronary BM-MNC treatment after AMI			
BOOST	n = 60	BM-MNC intracoronary, randomized	Safety+++
Wollert et al, Lancet 2004		6-m results	LVEF ⇑+
Meyer et al, Circ 2006		18-m results	LVEF ⇑−
Janssens et al	n = 67	BM-MNC intracoronary, randomized, double blind	Safety+++
Lancet 2006		Placebo controlled	LVEF ⇑−
REPAIR AMI	n = 201	BM-MNC intracoronary, randomized, double blind	Safety+++
Schächinger et al, NEJM 2006		Placebo controlled	LVEF ⇑+
ASTAMI	n = 100	BM-MNC intracoronary, randomized	Safety+++
Lunde et al, NEJM 2006			LVEF ⇑−
FINCELL study	n = 80	BM-MNC intracoronary, randomized	Safety+++
Huikuri et al, EHJ 2008			LVEF ⇑+
HEBE Trial	n = 200	Endothelial progenitor cells/BM-MNC intracoronary, randomized	Safety+++
Nijveldt et al, Circ 2008			LVEF ⇑−
Regent Trial	n = 200	BM-MNC/CD133+ intracoronary randomized	Safety+++
Tendera et al, EHJ 2009			VEF ⇑−

Despite continuous advances in the management of acute myocardial infarction (AMI), congestive heart failure secondary to ventricular remodeling after such an event continues to be a major medical problem worldwide.[1,2]

Progenitor cells hold a great potential for regenerative medicine, especially in replacing cells in tissues that hardly have any intrinsic regenerative capacity, including the heart. To date, 4 major questions remain still unresolved: (1) which type of progenitor cells is the most adequate for cellular therapy and how many cells have to be administered, (2) how to administrate the cells, (3) at what time point after AMI cell administration should be performed, and (4) are there criteria for patients qualifying most for cellular therapy?

Currently, the major source of different somatic cells used for basic research and for clinical trials in patients with acute or chronic cardiac ischemia is the bone marrow. Interestingly, experimental studies suggest that administration of progenitor cells deriving from bone marrow mononucleated cells(BM-MNCs) may regenerate infarcted myocardium and stimulate neovascularization of ischemic myocardium.[3,4] The safety and feasibility of intramyocardial and intracoronary application respectively of BM-MNC was demonstrated in first-in-man studies, with promising improvement of left ventricular (LV) function shortly after AMI.[5-8] Recently published double-blind placebo-controlled randomized clinical trials, however, did not reveal consistent results on improvement of LV function (Table I).[9-14]

The latter problem may be related to differences in cell preparation, study population, and different time point of treatment after AMI.

Perhaps the most promising data resulted from the REPAIR-AMI trial.[11] Injection of BM-MNC into the infarct-related coronary artery (IRA) resulted in a significant increase in left ventricular ejection fraction (LVEF) and beneficial effects on ventricular remodeling with particularly pronounced effects—as suggested by a subgroup analysis—in patients with reduced LVEF. Similar findings were made by Tendera et al,[14] who described a trend in
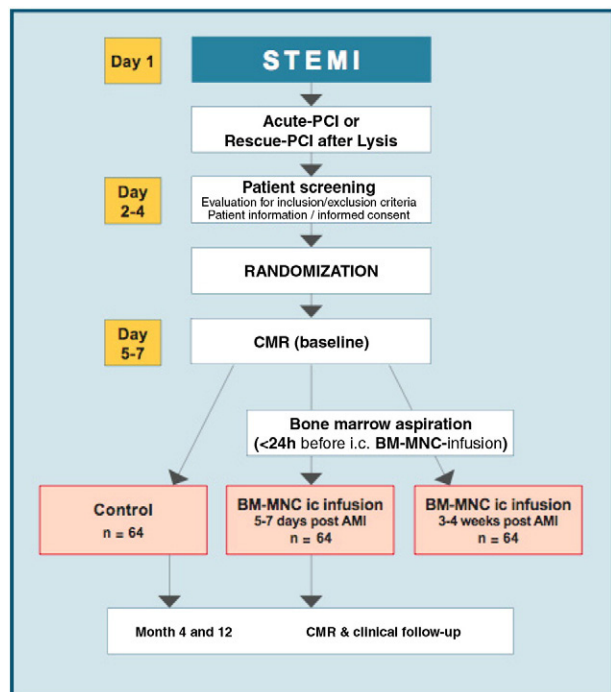
favor of BM-MNC therapy in patients with most severely impaired LVEF, whereas the study in general failed to demonstrate a positive effect of cell therapy on LV function.

In a subgroup analysis of a recent meta-analysis by Abel-Latif et al,[15] a more pronounced increase in LVEF was noted in patients treated with progenitor cell administration 5 to 30 days after AMI compared to those treated within the first 5 days. Especially in the REPAIR-AMI trial,[11] the beneficial effects of BM-MNC appeared to depend on the time of administration, although this again was apparent in a subgroup analysis only. Of note, however, very early administration (within the first 4 days) did not reveal a benefit over placebo, whereas a marked improvement of LVEF was seen if cells were infused between 4 and 8 days after AMI. The results of another randomized, double-blind, placebo-controlled trial in patients treated within 24 hours after AMI by Janssens et al[10] somewhat confirmed these findings. Likewise, despite significant reduction in infarct size and improvement in regional wall motion, no difference in LVEF was seen between patients treated with BM-MNC or placebo. A potential explanation for the blunted effects of BM-MNC in the acute setting of AMI might be related to a prooxidant and inflammatory local environment in the infarct area leading to local death of the injected cells or reduced engraftment.

In the randomized studies by Lunde et al[12] and in the HEBE trial,[16] no benefit of intracoronary BM-MNC infusion within 4 to 7 days after AMI on LVEF could be demonstrated. Interestingly, in both studies, the investigators used a different cell preparation technique that could have an influence on cell potency as suggested also by Seeger et al.[17]

Taken together, the clinical and preclinical trials published today provide limited data on the optimal time point of BM-MNC application and in turn for homing into the damaged myocardium. Preliminary preclinical data indicate that homing of BM-MNC is still increasing after 7 days. Thus, it appears mandatory to test in a

Figure 1

Flowchart study design.

Table II. Inclusion criteria

ST-segment elevation myocardial infarction with visual LVEF at angiogram or echocardiography ≤45% within 24 h after PCI of the IRA

Treatment by primary PCI within 24 h of the onset of chest pain or initial treatment with thrombolysis within the 12 h followed by PCI within 24 h of the onset of chest pain

Significant regional LV wall motion dysfunction in the infarct-related territory

Age >18 y

Table III. Exclusion criteria

Abnormal regional wall motion outside the infarct region

Known previous myocardial infarction in the same target vessel

Known preexisting LV dysfunction (ejection fraction <45% before admission)

Need for revascularization in the non–infarct-related coronary within 4 m

Preexisting symptoms of heart failure or known cardiomyopathy

Known active infection or chronic infection with HIV, hepatitis B virus, or hepatitis C virus

Chronic inflammatory disease

Serious concomitant disease with a life expectancy of <1 y

Follow-up unlikely (no fixed abode, and others)

Contraindication for CMR (ie, pacemaker, neurostimulator, severe claustrophobia)

Severe renal failure (creatinine level >250 mmol/L)

Relevant liver disease (aspartate aminotransferase >2× norm or spontaneous international normalized ratio >1.5)

Anemia (hemoglobin level <8.5 mg/dL), thrombocytopenia (<100,000/μL)

Pregnancy

Participation at a clinical trial in the last 30 d

prospective manner that delayed treatment with BM-MNC 3 to 4 weeks after AMI is still beneficial on LVEF as it is expected for early administration 5 to 7 days after AMI.

Another issue is the characterization of patients who will benefit the most from cellular therapy. Retrospective data from the REPAIR-AMI[11] and the REGENT study[14] favor patients with more severely impaired LV function. Furthermore, more insight into the effects of BM-MNC on transmural infarct extension and myocardial scar size would be important. Cardiac magnetic resonance (CMR) study using gadolinium delayed enhancement (DE) technique is considered as the gold standard in the assessment of LV scar mass after AMI as well as for characterization of its transmurality. Cardiac magnetic resonance study is further well established as a very reliable and reproducible diagnostic tool to assess LV volumes and function.

With the SWISS-AMI protocol, we hope to confirm the therapeutical benefit of intracoronary administration of autologous BM-MNC after AMI and aim to define the optimal time of cell therapy as well as clinical criteria for patients qualifying most for such therapy.

Methods

The study protocol was approved by the regional ethical committee of each participating center as well as by the Federal competent authorities.

The study is a multicenter, randomized, controlled evaluation of BM-MNC administration compared with standard therapy in patients with AMI and decreased LV function (LVEF <45%). The study design flowchart is summarized in Figure 1. Patients treated by successful primary percutaneous coronary intervention (PCI) of the IRA, fulfilling the inclusion criteria (Table II), and none of the exclusion criteria (Table III) are requested their informed consent to participate in the study. They are then randomized using closed envelopes in a 1:1:1 pattern in 2 therapy groups and 1 control group. The control group will receive best medical, whereas patients randomized to the therapy groups will be treated 5 to 7 days or 3 to 4 weeks, respectively, after AMI on top of standard therapy with intracoronary administration of BM-MNC. All 3 groups will undergo serial CMR studies at baseline (days 5-7) and at 4 and 12 months after AMI to assess the morphological and functional effects of intracoronary injection of BM-MNC.

Aim of the study

The primary objective of the study is to determine the effect of intracoronary administration of BM-MNC on LVEF in patients successfully treated by primary PCI after AMI. The primary end point is thus the change in global LVEF by CMR at 4 months compared to baseline. Comparisons will then be made between prespecified therapy subgroups and the control group to provide information in a prospective matter on the optimal time point for BM-MNC administration. To demonstrate

durability of treatment a further CMR study will be performed at 12 months after myocardial infarction.

Special focus will be held also on the analysis of predictors, which define the patients who will profit the most of cell treatment using the following secondary end points:

- change in infarct size by DE-CMR technique;
- change in regional myocardial thickness and wall motion at 4 and 12 months;
- infarct size, scar transmurality, time delay to PCI, and coronary flow characteristics after PCI will be analyzed as potential predictors of LV remodeling and change after cell therapy;
- major adverse cardiac events (MACE) (death, myocardial infarction, coronary revascularization, rehospitalization for heart failure) will be assessed as a combined end point at 4, 12, and 24 months; and
- time from randomization to first occurrence of a MACE.

Bone marrow aspiration and cell processing

Patients randomized to the treatment groups will undergo bone marrow aspiration within 24 hours before cell administration. For the latter, 50 mL of BM will be collected from the iliac crest under local anesthesia and then diluted in 1,000 IU/10 mL of heparin delivered by courier to a centralized cell processing facility (Cell Therapy Unit of the Cardiocentro Ticino, Lugano, Switzerland). The mononucleated cell fraction will be isolated according to a standard protocol deriving from previous studies with slight modifications.[18] Briefly, the bone marrow will be prefiltered, subjected to density gradient centrifugation on Ficoll Premium (GE Health care, Uppsala, Sweden). After several washings, the cells will be resuspended in 10 mL of X-VIVO 10 medium (Lonza, Walkersville, MD) added with 20% vol/vol of autologous serum and filled in syringes. An aliquot of cells will be collected for fluorescence-activated cell sorting analysis using directly conjugated antibodies against antihuman CD34, CD45, and CD133. Cell viability will be assessed by 7-aad cell uptake and product sterility by using BacT/ALERT PF bottles in the BacT/ALERT 3D rapid method (bioMérieux, Crappone, France). Mononucleated cells will be released only if cell number will be comprised between 5×10^7 and 5×10^8 and cell viability is ≥80%. Serology must be negative for hepatitis B surface antigen and for anti-HIV1/2 and antihepatitis C virus. The cells will be then retransferred by courier to the corresponding center that will administer the cells.

Intracoronary infusion of the BM-MNC

After puncture of the common femoral artery, patients will receive 5,000 IE of heparin, and a guiding catheter will be placed in the ostium of the former IRA. Cells will be infused via an over-the-wire balloon catheter advanced into the stent of the IRA and inflated with low pressure (2-4 bars) to completely block blood flow for 3 minutes to allow for adhesion and transmigration of the infused cells through the endothelium. This maneuver will be repeated 3 times to allow infusion of the total 9 mL of progenitor cell suspension, interrupted by 5 minutes of reflow by deflating the balloon to minimize extensive ischemia. After completion of intracoronary cell reinfusion, coronary angiography will be repeated to ascertain vessel patency, absence of embolization, and unimpeded flow of contrast material.

Periprocedural safety of the BM-MNC infusion will be monitored by assessment of serum cardiac enzymes including troponin the day after the intervention. Periprocedural myocardial infarction is defined as previously described.[19]

Cardiac magnetic resonance studies

Patients will undergo CMR studies at baseline, that is, 5 to 7 days after the occurrence of the AMI, as well as after 4 and 12 months of follow-up using 1.5T systems and dedicated cardiac coils in all centers. After localizer acquisitions, the CMR studies will include functional imaging of the left ventricle by means of standard electrocardiogram-triggered steady-state free precession acquisitions during repetitive breath holds in 3 long-axis orientations and in contiguous short-axis orientation covering the entire left ventricle. Viability/scar imaging by CMR is currently recognized as one of the most accurate techniques to quantify necrotic or fibrous tissue.[20,21] Therefore, in the second part of the CMR examination, contrast-enhanced inversion-recovery fast gradient echo imaging will be applied in combination with conventional extracellular Gd-chelates at a dose of 0.20 nmol/kg body weight (DE-CMR).[22,23] After determination of the inversion time nulling normal myocardium, scar imaging will be performed 20 minutes after administration of contrast medium in identical locations as functional data were acquired.

Cardiac magnetic resonance data analysis will be performed in a core laboratory (University Hospital Zurich/CH, Switzerland) using dedicated cardiac analysis software (GT-volume; Gyrotools Ltd, Zurich/CH, Switzerland). The LV end-diastolic and end-systolic volumes, LVEF, and LV mass will be quantified for assessment of the primary end point (change of LVEF) and for assessment of ventricular remodeling over time in the 3 groups. For functional analysis, systolic contraction will be graded in each segment as normal (score 0), mildly hypokinetic (= 1), severely hypokinetic (= 2), akinetic (= 3), or dyskinetic (= 4). The summed score will be calculated and divided by the total number of segments (which depends on the size of the heart). On the DE images, myocardial infarct size will be quantified using the full-width one half maximum method (Figure 2).[24,25] Transmurality of the scar tissue will also be visualized in polar map representations. The extent of microvascular obstruction delineated as dark areas in the core of the necrotic zone in the DE images will be quantified by contouring manually the dark core areas. Scar mass and tissue with microvascular obstruction will be expressed in grams and as a percentage of LV mass and of scar mass, respectively.

Changes over time (baseline, 4 months, 12 months) of volumetric, functional, and scar parameters will be compared between the groups to assess the influence of treatment on global and regional systolic function, LV remodeling, and scar mass.

Statistical analysis

Patients are considered entered in the trial and included in the analysis of safety in an intention-to-treat analysis when informed consent has been signed and the patient was randomized.

Descriptive statistics of continuous variables will be presented. Nominal variables are summarized in frequencies and percentages.

The statistical comparisons of the treatment arms with respect to the primary end point are performed using independent-

Figure 2
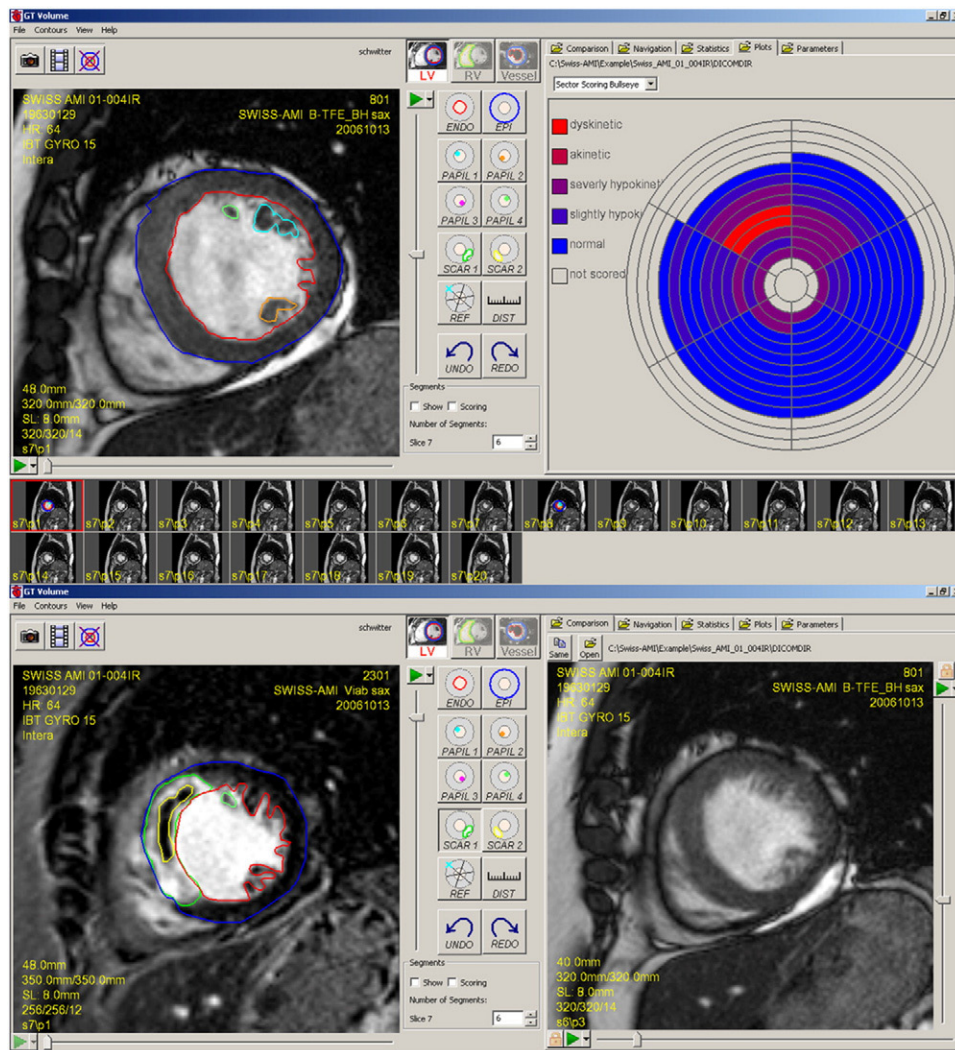
Example of the analysis of a CMR study giving results for volumetric, functional, and scar parameters. On the top, epicardial, and endocardial contours are shown including trabecular and papillary muscle mass. In this patients, LV parameters are LV end-diastolic volume, 119 mL; LV end-systolic volume, 48 mL; LV stroke volume, 71 mL; LVEF, 60.1%; and cardiac output, 4.6 L/min. Left ventricular mass measured at end-diastole was 142 g (at end-systole, 150 g). Functional score was 0.83 (49/59 segments) and is represented in the polar map on the right. On the bottom, the viability study is shown on the left, whereas the cine loop demonstrates function of the corresponding slice on the bottom right (visualized as a movie). Total scar mass of the left ventricle was 42 g, that is, 11.2 % of the necrotic mass.

samples t test. All P values are 2-sided. Patients with missing CMR end point measurements, as defined in the previous section, are left out (only) from the primary end point analyses. We account for a 10% dropout rate (see below).

For the 2 primary end point comparisons, namely, of both experimental arms to the standard, a P value of ≤.025 is considered as significant. Suitable 97.5% CIs for both differences of LVEF changes between treatment arms will be provided.

Supplementary analysis of covariance involving covariate adjustment, especially for baseline LVEF, is performed. Key baseline prognostic factors or confounding variables, that is, those where an imbalance might exist between treatment arms,

are included in these analyses. The influence of the total number and functionality of infused progenitor cells on LVEF changes and clinical outcome is analyzed by means of regression analyses with appropriate interpretative terms.

For the analysis of the secondary end points, all P values ≤.05 will be considered significant.

For the analysis of binary end points, comparisons will be performed using χ^2 or Fisher exact test, depending on the expected cell frequencies. For continuous outcomes, independent-samples t or Wilcoxon test are used.

The occurrence of MACE will be assessed and compared between groups at 4, 12, and 24 months. Because at time of

analysis not all patients will have had a MACE, the secondary end point time from randomization to MACE may be censored for these patients. Kaplan-Meier estimates of survival curves will be shown, and median time to MACE will be computed, including 95% CIs according to the method of Brookmeyer.[26] Estimated survival curves will be compared between groups using log-rank test.

Sample size calculation

The study is powered for the primary end point of the change in LVEF determined by CMR at 4 months compared to baseline.

We assumed an improvement in LVEF as assessed by quantitative MRI of 1% to 3% in the standard group and of 6% to 7% in 1 of the 2 treatment groups (according to recent studies[7,9,11]). This implies that we want to detect a difference between LVEF improvements from baseline to 4 months of $\delta =$ 3.5%. From the aforementioned previous studies, it is known that the SD of the difference baseline at 4 months approximately amounts to 6% to 7%. The primary question of the trial is the comparison of both treatments to the standard. To maintain the family wise error rate of $\alpha* = .05$, we therefore use the Bonferroni test to correct the significance level of the 2 tests each comparing a treatment arm to the control and compute a sample size for $\alpha = .025$. A sample size of n = 58 in each group will have 80% power to detect a difference in LVEF changes from baseline to 4 months between treatments of 3.5% assuming that the common SD is 6% using a 2-group t test with a 0.025 two-sided significance level. To account for a dropout of 10%, 6 additional patients should be recruited in each arm yielding for a sample size of n = 64 per arm and 192 for the entire trial.

"In vivo" and "in vitro" assessment of cell potency

To evaluate the cell functionality at the level of the myocardium, assays should be performed to estimate the intended biologic effect that should be related to the clinical response. The importance of characterizing the functionality of injected cells was recently pointed out by Dawn and Bolli[27] who proposed in vitro and in vivo assays. On the basis of the observation that the migratory capacity of BM-MNC predicts the functional improvement after cell transplantation in a hind limb ischemia model[28] and in humans,[29] we chose cell migration and invasion assays (as previously described[30]) as in vitro tests for cell potency. Furthermore, in vivo tests will be performed by using a nude mice model of myocardial infarction (as previously described[31]). Two weeks post-AMI, the effect of cell administration will be characterized by CMR, hemodynamic measurements, and histologic analyses. Furthermore, the capillary density and the amount of apoptotic cells in the border infarct zone will be evaluated by immunofluorescence. Myocyte cross sectional area and interstitial fibrosis will be also evaluated. Cell homing will be addressed by specific labeling for human antigens (such as anti-HLA).

Discussion

Until present, most of the published clinical studies focused on BM-MNC administration during the first week after AMI. Despite preliminary data, it is still not clear if the time of administration plays an important role, and therefore, the optimal time for administration remains elusive and a matter of extensive discussion.[32] It is likely that the microenvironment within the infarct tissue and the timing of cell delivery may be an important determinant of cell incorporation and fate. The REPAIR-AMI study[11] disclosed that intracoronary BM-MNC infusion within 4 days after reperfusion therapy for AMI only had marginal effects on recovery of LV contractile function. It is tempting to speculate that early after infarct reperfusion therapy, the presence of tissue edema and toxic reperfusion products may provide a "hostile" environment, which may limit potential effects of progenitor cells administered.

Here, we present and discuss the rationale and study design of a multicenter, randomized, controlled protocol to evaluate the benefit of intracoronary BM-MNC transplantation compared with standard therapy in patients with AMI. The main goal of the study consists in defining the optimal time point of cell administration as well as in the research of clinical predictors for patients having the largest benefit of cell therapy. We therefore designed a trial with 2 BM-MNC treatment groups. One group will receive intracoronary administration of BM-MNC at 5 to 7 days after AMI, and the other group will receive the BM-MNC at 3 to 4 weeks. This group will provide essential information on the quality of BM-MNC and their homing potential at late administration.

The use of CMR to evaluate the effect of the treatment will allow the definition of predictors for ventricular remodeling and for a beneficial effect of progenitor cell-based therapy. We expect that patients with complete transmural extension of the myocardial infarction be at high risk for negative remodeling (mostly resulting in dilatation of the left ventricle leading to the clinical entity of heart failure): those patients should, in our opinion, have the great benefits from a cell-based therapy. The results will hopefully have important clinical impact and provide essential information to improve the design of future regenerative medicine protocols in cardiology.

As a potential limitation of the study, it has to be pointed out that the tested treatment regimen consists in unselected mononuclear cells and therefore reflects only one aspect of a growing variety of different cell therapy modalities. Future studies have to show if stimulation or "treatment" of the bone marrow may enhance function or density of the BM-MNC or if other cell types such as induced pluripotent stem cells will have stronger effects on myocardial regeneration. Other important questions such as the influence of or whether allogenic stem cells could be used for the same purpose[33] cannot not be addressed in the presented study.

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References

1. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 2003;348:1309-21.
2. Pfeffer MA, McMurray JJ, Velazquez EJ, et al. Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. N Engl J Med 2003;349:1893-906.
3. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-5.
4. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430-6.
5. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913-8.
6. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002;106:3009-17.
7. Schachinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. J Am Coll Cardiol 2004;44:1690-9.
8. Fernandez-Aviles F, San Roman JA, Garcia-Frade J, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circ Res 2004;95:742-8.
9. Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004;364:141-8.
10. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 2006;367:113-21.
11. Schachinger V, Erbs S, Elsasser A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 2006;355:1210-21.
12. Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355:1199-209.
13. Huikuri HV, Kervinen K, Niemela M, et al. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 2008;29:2723-32.
14. Tendera M, Wojakowski W, Ruzyllo W, et al. Intracoronary infusion of bone marrow-derived selected CD34$^+$CXCR4$^+$ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. Eur Heart J 2009;30:1313-21.
15. Abdel-Latif A, Bolli R, Tleyjeh IM, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 2007;167:989-97.
16. Nijveldt. Intracoronary Infusion of Mononuclear Cells After Primary Percutaneous Coronary Intervention: The HEBE Trial. Circulation 2008;118:2309-17 (2008 Late-Breaking Clinical Trial Abstracts).
17. Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S. Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. Eur Heart J 2007;28:766-72.
18. Schachinger V, Tonn T, Dimmeler S, Zeiher AM. Bone-marrow-derived progenitor cell therapy in need of proof of concept: design of the REPAIR-AMI trial. Nat Clin Pract 2006;3(Suppl 1):S23-8.
19. Thygesen K, Alpert JS, White HD, et al. Universal definition of myocardial infarction. Circulation 2007;116:2634-53.
20. Schwitter J, Saeed M, Wendland MF, et al. Influence of severity of myocardial injury on distribution of macromolecules: extravascular versus intravascular gadolinium-based magnetic resonance contrast agents. J Am Coll Cardiol 1997;30:1086-94.
21. Rehwald WG, Fieno DS, Chen EL, Kim RJ, Judd RM. Myocardial magnetic resonance imaging contrast agent concentrations after reversible and irreversible ischemic injury. Circulation 2002;105:224-9.
22. Knuesel PR, Nanz D, Wyss C, et al. Characterization of dysfunctional myocardium by positron emission tomography and magnetic resonance: relation to functional outcome after revascularization. Circulation 2003;108:1095-100.
23. Kim RJ, Wu E, Rafael A, et al. The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. N Engl J Med 2000;343:1445-53.
24. Schmidt A, Azevedo CF, Cheng A, et al. Infarct tissue heterogeneity by magnetic resonance imaging identifies enhanced cardiac arrhythmia susceptibility in patients with left ventricular dysfunction. Circulation 2007;115:2006-14.
25. Amado LC, Gerber BL, Gupta SN, et al. Accurate and objective infarct sizing by contrast-enhanced magnetic resonance imaging in a canine myocardial infarction model. J Am Coll Cardiol 2004;44:2383-9.
26. Brookmeyer R. A confidence interval for the median survival time. Biometrics 1982;38:29-41.
27. Dawn B, Bolli R. Bone marrow for cardiac repair: the importance of characterizing the phenotype and function of injected cells. Eur Heart J 2007;28:651-2.
28. Heeschen C, Lehmann R, Honold J, et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation 2004;109:1615-22.
29. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation 2003;108:2212-8.
30. Soncin S, Lo Cicero V, Astori G, et al. A practical approach for the validation of sterility, endotoxin and potency testing of bone marrow mononucleated cells used in cardiac regeneration in compliance with good manufacturing practice. J Transl Med 2009;7:78.
31. Doerries C, Grote K, Hilfiker-Kleiner D, et al. Critical role of the NAD (P)H oxidase subunit p47phox for left ventricular remodeling/dysfunction and survival after myocardial infarction. Circ Res 2007;100:894-903.
32. Bartunek J, Wijns W, Heyndrickx GR, Vanderheyden M. Timing of intracoronary bone-marrow-derived stem cell transplantation after ST-elevation myocardial infarction. Nat Clin Pract 2006;3(Suppl 1):S52-6.
33. Hare JM, Traverse JH, Henry TD, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 2009;54:2277-86.