

LateTIME

A Phase-II, Randomized, Double-Blinded, Placebo-Controlled, Pilot Trial Evaluating the Safety and Effect of Administration of Bone Marrow Mononuclear Cells 2 to 3 Weeks after Acute Myocardial Infarction

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A realistic goal for cardiac cell therapy may be to attenuate left ventricular remodeling following acute myocardial infarction to prevent the development of congestive heart failure. Initial clinical trials of cell therapy have delivered cells 1 to 7 days after acute myocardial infarction. However, many patients at risk of developing congestive heart failure may not be ready for cell delivery at that time-point because of clinical instability or hospitalization at facilities without access to cell therapy. Experience with cell delivery 2 to 3 weeks after acute myocardial infarction has not to date been explored in a clinical trial. The objective of the LateTIME study is to evaluate by cardiac magnetic resonance the effect on global and regional left ventricular function, between baseline and 6 months, of a single intracoronary infusion of 150×10^6 autologous bone marrow mononuclear cells (compared with placebo) when that infusion is administered 2 to 3 weeks after moderate-to-large acute myocardial infarction. The 5 clinical sites of the Cardiovascular Cell Therapy Research Network (CCTRN) will enroll a total of 87 eligible patients in a 2:1 bone marrow mononuclear cells-to-placebo patient ratio; these 87 will have undergone successful percutaneous coronary intervention of a major coronary artery and have left ventricular ejection fractions ≤ 0.45 by echocardiography. When the results become available, this study should provide insight into the clinical feasibility and appropriate timing of autologous cell therapy in high-risk patients after acute myocardial infarction and percutaneous coronary intervention. (Tex Heart Inst J 2010;37(4):412-20)

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Following an acute myocardial infarction (AMI), there is replacement of myocytes by fibrotic tissue and ongoing apoptotic loss of viable cardiac myocytes in the infarct border zone as a result of reperfusion injury and ongoing ischemia from microvascular obstruction. If the infarction is large, left ventricular (LV) dysfunction may develop due to scar expansion and LV dilation, ultimately leading to the development of congestive heart failure. Congestive heart failure is the leading admission diagnosis for hospitalization in the United States and carries a 5-year mortality rate of 50%.¹ Although medical therapy can improve symptoms and extend survival to a limited degree, cardiac transplantation remains the only curative procedure available.

Recent studies in animals with experimental AMI have observed significant recovery of LV function after the delivery of bone marrow-derived stem cells.²⁻⁴ Although the mechanisms of benefit are subject to ongoing investigation,^{5,6} the potential has led to the initiation of multiple clinical trials to test the concept that delivery of autologous bone marrow mononuclear cells (BMMNCs) into the infarct region can improve cardiac function after AMI.⁷⁻¹¹ Several meta-analyses¹²⁻¹⁴ have demonstrated that

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BMMNC administration results in a small, but significant, improvement in LV function and attenuation of adverse LV remodeling.

The timing of cell administration after AMI may be of fundamental importance in determining the success of cell therapy in this patient population, given the temporal changes that occur in the myocardium in the days to weeks after AMI that can affect stem cell survival and efficacy. Initial up-regulation of chemokine production, such as stromal derived factor-1 (SDF-1), can direct cell homing and differentiation,¹⁵ while stem cell-secreted growth factors such as vascular endothelial growth factor (VEGF) and insulin growth factor-1 can improve perfusion (through angiogenesis) and reduce apoptotic cell death in the infarct border zone.¹⁶ However, the development of a vigorous inflammatory response, coupled with the release of reactive oxygen species and cytokines such as tumor necrosis factor- α in the infarct region after an AMI, can adversely affect cell survival¹⁷ and limit the efficacy of this therapeutic approach.

No clinical trial published to date has been sufficiently powered to adequately answer questions about the efficacy of late cell delivery after AMI. Recently, the National Heart, Lung and Blood Institute (NHLBI) established the Cardiovascular Cell Therapy Research Network (CCTRN) to answer important questions in cell therapy, such as the optimal timing of cell delivery after AMI. The 1st TIME trial developed by the CCTRN is currently investigating outcome differences in LV function in patients randomized to cell delivery at 3 versus 7 days after AMI.¹⁸ However, many patients eligible for cell therapy may not be suitable for randomization at these time-points, due to clinical instability or to hospitalization at facilities without access to cell therapy. To study the usefulness of cell therapy in this important subset of AMI patients, the CCTRN developed a 2nd randomized, placebo-controlled trial called LateTIME, in which autologous BMMNCs are delivered 2 to 3 weeks after AMI.

Organizational Structure and Oversight

The CCTRN was established by the NHLBI to develop, coordinate, and conduct multiple collaborative protocols testing the effects of stem cell therapy on cardiovascular disease. The Network builds on contemporary findings of the cell-therapy basic science community, translating this newly acquired information to the cardiac clinical setting in the Phase I/II study paradigm. The Network consists of 5 clinical research centers (Cleveland Clinic Foundation, University of Florida, Minneapolis Heart Institute/University of Minnesota, Texas Heart Institute, and Vanderbilt University), a data coordinating center (DCC) (University of Texas School of Public Health) that provides trial management and data analysis, a cell-processing quality-control center, and 5 core laboratories. Together, these Network components

provide standardization of cell-therapy preparation and endpoint measurements. All clinical centers participate in the selection and design of Network protocols, which are also reviewed by an independent Protocol Review Committee and a Gene Therapy/Cell Therapy Data Safety and Monitoring Board (DSMB), under the aegis of the NHLBI. Each clinical center and the DCC has independent institutional review board (IRB) approval and oversight. By recruiting from multiple centers, the Network accelerates the speed with which its studies can be completed, increases the broad applicability of study findings, and amplifies the dissemination of its public health findings.

Objectives and Design

The primary objective of LateTIME, a Phase-I/II, randomized, double-blinded, placebo-controlled clinical trial, is to determine whether delayed (2–3 wk) administration of intracoronary autologous BMMNCs to patients after a moderate-to-large MI can safely produce a measurable improvement in LV global and regional function as determined by cardiovascular magnetic resonance (CMR) at 6 months, compared with baseline. Secondary objectives are to determine whether the administration of cell therapy in comparison with placebo will produce smaller end-diastolic and end-systolic volumes at 6 months, compared with baseline. Secondary objectives are to determine whether the administration of cell therapy in comparison with placebo will produce smaller end-diastolic and end-systolic volumes at 6 months. Patients will be monitored for 2 years to evaluate the effects of cell therapy on the clinical endpoints of death, reinfarction, repeat target-vessel revascularization, hospitalization for congestive heart failure, and placement of implantable cardiac defibrillators (ICDs). The effects of cell phenotype on therapeutic efficacy will be investigated through ancillary studies at 2 biorepository core facilities (the University of Minnesota and the University of Florida, Gainesville).

Patients will be randomized in a 2:1 ratio, BMMNC to placebo. The overall anticipated sample size required for LateTIME is 87 patients (58 cell therapy and 29 placebo). Hypothesis testing for the primary outcome will be carried out at the 0.05 level.

Enrollment and Study Population

The 87 patients required for LateTIME will be recruited from all sites participating in the NHLBI CCTRN and their developed satellite referral centers. Each patient will have undergone successful percutaneous revascularization of a major coronary artery after a moderate-to-large infarction with a left ventricular ejection fraction (LVEF) ≤ 0.45 as evaluated by echocardiography. All prospective patients will be screened by investigators or study coordinators and will be enrolled in the trial after meeting inclusion criteria (Table I) and signing

the informed consent and Health Insurance Portability & Accountability Act forms.

Eligibility and Randomization

Once inclusion and exclusion criteria have been evaluated and informed consent has been obtained, eligible patients will be randomly assigned to one of the selected treatment strategies in an interactive Web-based randomization session. Patients will be randomized in a 2:1 (BMMNC-to-placebo) ratio using randomly selected block sizes of 6 or 9 and stratified by the center. The patients and research staff, including the CCTRN physicians and interventional cardiologists, will be blinded to treatment assignment. Patients will follow a prescribed

schedule of procedures (Table II) and be evaluated at 6 months for efficacy of treatment. All patients will be monitored for 2 years to obtain an extensive safety profile.

Bone Marrow Aspiration

All patients will receive a physical examination, including evaluation of vital signs, height, weight, and New York Heart Association (NYHA) functional class. On the morning of study product infusion, patients will undergo bone marrow aspiration in accordance with standard operating procedures developed by the CCTRN. Patients will be lightly sedated or receive conscious sedation as determined by the attending physician. Approx-

TABLE I. Major Inclusion and Exclusion Criteria

1. Inclusion

- a) Patients at least 21 years of age.
- b) Patients with 1st acute myocardial infarction (AMI) who undergo, 2 to 3 weeks before recruitment, successful primary percutaneous coronary intervention (PCI) in a coronary artery that is at least 2.5 mm in diameter.
- c) No contraindications to undergoing cell-therapy procedure within 2 to 3 weeks after AMI and PCI.
- d) Hemodynamic stability—defined as no requirement for intra-aortic balloon pump or for inotropic or blood-pressure supporting medications.
- e) Left ventricular ejection fraction (LVEF) ≤ 0.45 after reperfusion with PCI, as determined by echocardiography.
- f) Consent to protocol and agree to comply with all follow-up visits and studies.
- g) Women of child-bearing potential willing to use an active form of birth control.

2. Exclusion

- a) History of sustained ventricular arrhythmias not related to patients' myocardial infarction (evidenced by previous Holter monitoring and/or medication history for sustained ventricular arrhythmias in patient's medical chart).
 - b) Require coronary artery bypass grafting (CABG) or PCI due to the presence of residual coronary stenosis $>70\%$ luminal obstruction in the noninfarct-related vessel (additional PCI of non-culprit vessels may be performed prior to enrollment).
 - c) History of any malignancy within the past 5 years, excluding non-melanoma skin cancer or cervical cancer in situ.
 - d) History of chronic anemia (hemoglobin, <9 g/dL).
 - e) History of thrombocytosis (platelets, $>500,000/\text{mm}^3$).
 - f) Baseline platelet count (before revascularization) $<120,000/\text{mm}^3$ or known history of thrombocytopenia.
 - g) Known history of elevated international normalized ratio (prothrombin time) or partial thromboplastin time.
 - h) Life expectancy of less than 1 year.
 - i) History of untreated alcohol or drug abuse.
 - j) Currently enrolled in another investigational drug or device trial.
 - k) Previous CABG.
 - l) Previous myocardial infarction resulting in left ventricular dysfunction (LVEF, <0.55).
 - m) History of stroke or transient ischemic attack within the past 6 months.
 - n) History of severe valvular heart disease (aortic valve area, <1 cm^2 ; or grade $>3+$ mitral regurgitation).
 - o) Pregnancy or breastfeeding.
 - p) Subjects who are tuberculosis positive or have a known history of human immunodeficiency virus, hepatitis B, or hepatitis C infection.
 - q) Patients with active inflammatory or autoimmune disease on chronic immunosuppressive therapy.
 - r) Contraindications to cardiovascular magnetic resonance.
 - s) Previous radiation to the pelvis with white blood cell count and platelet counts below hospital-specific normal values.
 - t) Women of child-bearing potential not willing to practice an active form of birth control.
 - u) Chronic liver disease that might interfere with survival or treatment with cell therapy.
 - v) Chronic renal insufficiency as defined by a creatinine level ≥ 2 mg/dL or by the need for chronic dialysis.
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imately 80 to 90 mL of bone marrow will be aspirated from the iliac crest using standard techniques. Only one bone marrow aspiration will be attempted. As determined from preclinical experience, 150 to 200 million total nucleated cells (TNCs) can be routinely harvested with this volume of bone marrow aspirate, containing small fractions of CD34⁺ and CD133⁺ cells. The specific population of cells administered in LateTIME will be determined at the biorepository core facilities and correlated with outcomes.

Cell Preparation and Cell Product

Once harvested, the cells will be transported to the institution's cell-therapy laboratory. Each site will use the

Sepax[®] system¹⁹ (Biosafe America, Inc.; Houston, Tex) for BMMNC isolation. The Sepax system is a closed system that enables faster isolation of the cellular product and, potentially, increased safety for the patient. Each site's cell-therapy laboratory director and participating personnel have been trained with the Sepax system, thereby ensuring a more uniform cellular product. The cells will be harvested and washed 3 times in human serum albumin/saline buffer before re-suspension in 5% human serum albumin/saline. The composition of CD34⁺ and CD133⁺ cells will be determined by fluorescent-activated cell-sorting analysis. Viability of the cells will be determined by trypan blue exclusion; ≥70% viability will be required before transplantation. A 14-day

TABLE II. Schedule of Procedures in LateTIME

	Consent (2–3 wk post-AMI)	Day 0 (SPI)	Day 1	Month				
				1	3	6	12	24
Complete medical history	X							
Inclusion/exclusion criteria review	X							
Incremental medical history		X	X	X	X	X	X	X
Informed consent	X							
Physical examination ¹	X	X	X	X	X	X	X	X
NYHA class assessment	X		X	X	X	X	X	X
Laboratory tests	X ²		X ³	X ⁴	X ⁴	X ⁶	X ⁶	X ⁶
Echocardiogram	X				X ⁵			
Electrocardiogram (12-lead)	X		X	X		X		
Bone marrow aspiration ⁷		X						
Biorepository blood draw ⁸		X	X			X		
Cardiovascular magnetic resonance		X				X	X	X
Study product infusion		X						
Medication review	X	X	X	X	X	X	X	X
AE/SAE assessment		X	X	X	X	X	X	X
Telemetry (18–24 hr post-infusion)		X	†					
Holter (24-hr)				X				

AE/SAE = adverse event/severe adverse event; AMI = acute myocardial infarction; NYHA = New York Heart Association; SPI = study product infusion

¹Including vital signs, height, and weight

²Complete blood count/differential, lipid panel, renal panel, hepatic panel, troponin T or I, creatine kinase, creatine kinase–MB fraction, high-sensitivity C-reactive protein, brain natriuretic peptide level, and pregnancy test (women of child-bearing age)

³Complete blood count/differential, renal and hepatic panels, and troponin T or I, creatine kinase, creatine kinase–MB fraction, collected every 6 to 8 hours post-infusion

⁴Complete blood count/differential, hepatic panel, and pregnancy test (women of child-bearing age)

⁵Limited

⁶Complete blood count/differential, hepatic panel, and pregnancy test (women of child-bearing age), brain natriuretic peptide

⁷Ascertainment of pre- and post-procedural vital signs

⁸30 mL venous, buffy coat

†Assessment of the earlier 18 to 24 hours of telemetry

sterility culture will be performed on the final product. Because this testing will not be available before product infusion, a negative Gram stain will be required before the product is released. Although cells are autologous in this protocol, the standard tests for infectious diseases will be performed. Additional testing deemed necessary by regulations or institutional policy (or both) will be performed. Final product (lot) release criteria testing results (Table III) will be available before transport of BMMNCs to the hospital for administration. The product will be labeled and tracked with adhesive labels containing the patient's study identification.

All patients randomized to the active group will receive 150×10^6 TNCs (70%–80% BMMNCs) as determined by a hematology analyzer. If the bone marrow aspiration yield is less than the target amount and the patient has already been randomized to therapy, then he or she will receive all available cells. Patients randomized to the placebo group will receive 5% albumin in normal saline, to which 100 μ L of autologous blood will be added to ensure that the color and consistency of the placebo infusion matches that of the active group. If the patient consents, all cells not required for study product delivery will be provided to the CCTRN biorepository core facilities for cell characterization and functional analysis.

Infusion of Cellular Product or Placebo

The final cellular product or placebo (approximate total volume, 30 mL) will be infused less than 12 hours after bone marrow aspiration. The patients will be heparinized to an activated clotting time of >200 sec, and the infusate will be delivered via an over-the-wire percutaneous transluminal coronary angioplasty catheter (stop-flow) in 6 aliquots of approximately 5 mL each, delivered over 2 minutes of balloon inflation within the previously placed stent at the lowest possible pressure necessary to obstruct coronary blood flow (3–6 atm). Two minutes of reperfusion will occur after each cycle of cell infusion.

All patients will be monitored overnight on a telemetry unit in the hospital and seen by a physician the fol-

lowing morning, before discharge. Patients are advised to take aspirin for life and 75 mg of clopidogrel for 24 months, as well as the usual medications for post-AMI care. Patients with LVEFs of <0.40 will be advised to take an aldosterone antagonist unless contraindicated by a creatinine level ≥ 2.5 mg/dL or a serum potassium level ≥ 5 mEq/L.

Determination of Outcomes

A 1.5T CMR scanner will be used, with precise magnetic resonance imaging (MRI) protocols developed by the MRI core laboratory (University of Florida). Cardiovascular Angiography Analysis System/Magnetic Resonance Ventricular (CAAS/MRV) cardiac function and perfusion analysis software (PIE Medical Imaging B.V.; Maastricht, The Netherlands) will be used to measure global LV myocardial mass, volumes, and LVEF, as well as regional parameters. Regional systolic wall motion, ejection fraction, thickening, and radial displacement in the infarct and border zones will be determined. Regional ejection fraction will be calculated for the basal, midwall, and apical portions of the LV cavity. Areas of microvascular obstruction, infarct size, and degree of transmuralty will be quantified by delayed gadolinium-enhanced MRI.

Wall Motion Imaging. Data on both global and segmental LV function will be obtained using a steady-state free-precession or fast-gradient echo technique. Long-axis cine images in the 2-chamber and 4-chamber projections will be acquired. In addition, a set of contiguous short-axis slices (8–10 mm thick) will be obtained from the mitral valve annulus through the apex of the LV throughout the cardiac cycle. Data will be analyzed using the CAAS/MRV software. Global measurements evaluated will include end-diastolic volume, end-systolic volume, stroke volume, ejection fraction, and LV mass. Volumetric measurements will be performed by direct planimetry on the contiguous short-axis images at both end-systole and end-diastole. Regional measurements will include wall thickening and wall motion, and will

TABLE III. Final Product Release Criteria Testing

Criterion	Assay	Test Method	Specification
Product release specifications	Rapid sterility	<ul style="list-style-type: none"> • Gram stain • Endotoxin 	No organisms
	Viability	Trypan blue	$\geq 70\%$
	Endotoxin	Endosafe®-PTS™	<5 EU/kg
	TNC	Manual or automated	$\leq 150 \times 10^6$
Post-production monitoring	<ul style="list-style-type: none"> • Immunophenotyping • Colony-forming units • Sterility 	<ul style="list-style-type: none"> • Flow cytometry • Per site SOP • 14-day culture 	<ul style="list-style-type: none"> • Report • Report • No growth

SOP = standard operating procedure; TNC = total nucleated cell

The Endosafe®-PTS™ (Charles River Laboratories International, Inc.; Wilmington, Mass) is an endotoxin-detection product.

be calculated using 100 chords spaced every 3.6°, originating from the centroid of the LV. Regional data will be reported using the American Heart Association 17-segment model.²⁰ The minimum spatial and temporal resolution requirements of the steady-state free-precession sequence are 2.5 × 2.5-mm voxels and 40 ms, respectively.

Baseline Perfusion Imaging. A 2-chamber long-axis cine image will be obtained on the basis of axial scout images, with the imaging plane spanning the center of the mitral valve coaptation point and through the apex of the LV. Based on this, a 4-chamber long-axis cine image will be obtained. Subsequently, a T₁-weighted gradient-echo baseline perfusion sequence will be performed using an intravenous gadolinium chelate (for example, gadolinium-DTPA). Three short-axis slices will be obtained (positioned from the 2-chamber and 4-chamber cine images) to encompass the basal, middle, and apical thirds of the LV during a bolus administration of gadolinium chelate (0.15–0.2 mmol/kg). Imaging will be acquired for a total of 60 dynamics per slice, ensuring that the passage of contrast material through the myocardium is captured for semiquantitative analysis.

Viability Imaging. Fifteen to 20 minutes after the administration of gadolinium-chelate contrast agent, delayed-enhancement imaging (DE-MRI) will be performed with a T₁-weighted inversion-recovery prepared gradient-echo sequence. The inversion delay time will be iteratively adjusted for optimal nulling of normal myocardium. Contrast-enhanced viability imaging will be performed with 2 techniques: the standard 2-dimensional technique, which acquires a single slice during each breath hold, will be performed in the short-axis projections, using the same plane prescription as the functional short-axis cine series; and a high-resolution 3-dimensional technique will be used to acquire 10 short-axis slices during a single breath hold. Regions of irreversible myocardial damage are manifested by “hyperenhancement” (bright white areas) on the images, while normal or viable tissue is “nulled” (black) on the acquired images. The presence, location, and extent of irreversibly damaged tissue will be qualitatively and quantitatively evaluated on a segmental basis. Pre- and post-therapy imaging, both cine wall motion and DE-MRI, will be carefully matched for consistency and accuracy, using internal landmarks that include the insertion sites of the right ventricular free wall and the papillary muscles.

Safety Monitoring

All CCTRN participants will be closely monitored for adverse events, and this information will be transmitted to the IRBs of all centers, the U.S. Food and Drug Administration, and the DSMB through the DCC. The DSMB will meet at least twice yearly to review the performance of the participating sites, to evaluate the ac-

cruing safety data, and to ascertain the feasibility of continuing the study. A set of stopping rules has been developed in consultation with the DSMB.

In addition, the DCC will oversee and coordinate the collection, standardization, integration, and analysis of study data from the various study components (enrolling sites and core facilities) and the preparation and distribution of the required reports to each of the safety-oversight entities. The DCC will facilitate and monitor regulatory and safety compliance at each site and core laboratory and will conduct site visits to each site and core laboratory to ensure protocol adherence and regulatory compliance, both on a regular basis and for cause.

Statistical Analyses

Assuming the independence and normalcy of the observations, we calculated the sample size using a 2-sample *t*-test statistic. The literature²¹ suggests that the absolute change (effect size, δ) achievable in global LVEF is $\delta=4\%$ and that the common group SD of the difference of LVEF over time is $\sigma_{\Delta}=6\%$. These produced a study sample size of 86, administratively rounded up to 87, with 58 participants in the active group and 29 in the control group. For a regional assessment of left ventricular function, we are assuming an absolute change in regional ejection fraction of $\delta=6.7\%$ and a common group SD of $\sigma_{\Delta}=9.5\%$, from the 2004 BOOST clinical trial report⁷; these assumptions produced a sample size of 77 patients. On the basis of the assumptions described above for global and regional LV function, the overall anticipated sample size required for LateTIME is 87 patients.

Exact testing for categorical variables and Student's *t*-testing for continuous variables will be used to evaluate the compatibility of baseline variables between treatment groups. All hypothesis testing, and all effect sizes and their 95% confidence intervals, will be evaluated using the general linear mixed model. Both unadjusted and adjusted treatment effects will be computed for primary and secondary endpoints (Table IV); adjustments will be for clinical centers, as well as for baseline covariates whose association with the dependent variable is generally accepted. In keeping with standard methodology for clinical trials, the primary and secondary comparisons will compare the randomized study groups using an intention-to-treat analysis. Logistic regression will be used to evaluate the effect of cell administration on the combined endpoint of death, reinfarction, repeat target-vessel revascularization, hospitalization for congestive heart failure, and ICD placement. No adjustments for multiple comparisons will be made.

Discussion

To date, more than 1,000 patients with AMI have been treated with intracoronary BMMNCs throughout the

TABLE IV. Outcomes in LateTIME; Active versus Placebo Comparisons

Outcome	Measurement	Method of Measurement
I. CO-PRIMARY	Δ Global LV function	CMR
	Δ Regional LV function	CMR
II. SECONDARY	A. Composite of: Death	Clinical outcome
	Reinfarction	Clinical outcome
	Repeat revascularization	Clinical outcome
	Hospitalization for HF	Clinical outcome
	ICD placement	Clinical outcome
	B. LV mass*	CMR
	C. LVEDV*	CMR
	D. LVESV*	CMR
	E. Infarct size	CMR
III. Additional/Subgroups	Age, sex, race, hypertension history, diabetes mellitus, statin use, DES/BMS use	Baseline characteristic
	Microvascular obstruction	CMR

BMS = bare-metal stent; CMR = cardiovascular magnetic resonance; DES = drug-eluting stent; HF = heart failure; ICD = implantable cardioverter-defibrillator; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume

*Normalized to body surface area

world. In total, studies¹²⁻¹⁴ show that cell therapy appears safe over several years of follow-up and results in a small, but significant, improvement in LV function and remodeling. However, fundamental questions in cell therapy, such as dose, optimal cell type, and timing of administration after AMI, have not been answered. The CCTRN was established by the NHLBI to develop protocols to answer these important questions in cell therapy.

In the previously published randomized clinical trials,⁷⁻¹¹ BMMNCs were administered between 1 and 7 days, but timing was never integrated into the randomization scheme; therefore, the effect of timing of BMMNC administration after AMI is not known. The 1st protocol developed by the CCTRN, called TIME,¹⁸ is a randomized, double-blinded, placebo-controlled trial that is evaluating the optimal time-point to deliver BMMNCs after a moderate-to-large MI. In that study, patients are randomized to cell therapy at either day 3 or day 7 after MI. This 2nd protocol developed by the CCTRN, LateTIME, will continue the investigation of the important aspect of cell-delivery timing. LateTIME is a randomized, double-blinded, placebo-controlled trial that will investigate the potential benefit of BMMNC administration 2 to 3 weeks after AMI in high-risk patients with persistent LV dysfunction. Evaluation of this time-point is important for many patients who are too ill to undergo randomization in a cell-therapy trial during the 1st few days after AMI, or who are hospitalized at centers that do

not have access to cell therapy, but may present to cell-therapy centers weeks later.

The timing of cell delivery after AMI may be one of the most important criteria in determining the efficacy of cell therapy. After AMI, a confluence of changes occurs in the myocardium; these changes include the expression of growth factors and cytokines, which might promote cell survival and angiogenesis or, conversely, might encourage myocyte apoptosis and adverse LV remodeling. Expression of chemokines such as SDF-1,¹⁵ which may aid in stem cell homing, is up-regulated in the infarct zone in the 1st few days after AMI. On the other hand, a strong inflammatory reaction and release of reactive oxygen species in the infarct zone may adversely affect the survival of injected cells.¹⁷ In a pre-clinical model, Ma and colleagues²² administered 5×10^6 mesenchymal stem cells (MSCs) via the tail vein in rats at multiple time-points after AMI (12 hr, and 1, 2, 4, 8, and 16 d). They observed that the greatest number of labeled MSCs retained in the heart when measured 3 days after administration occurred in those animals that received cells 1 day after AMI. The number of retained cells declined significantly with each subsequent day of administration so that no cells were present when cell administration occurred at 8 or 16 days after AMI. These observations are consistent with recent clinical findings that homing of radiolabeled progenitor cells to the infarct zone are greatest in the 1st few days after AMI.²³ These findings suggest that delayed administration of stem cells (up to several weeks after an AMI)

may not be as effective as earlier administration. However, several considerations—other than the fact that the patients of LateTIME are ineligible for earlier administration—justify exploring this later time-point. These include the roles of ongoing apoptosis during the first several months after AMI,²⁴ ongoing resolution of microvascular obstruction (which might improve intracoronary cell delivery to the infarct zone), and the abatement of a vigorous inflammatory response (which can affect stem cell survival).²⁵ Furthermore, all previous trials in which cells have been delivered within the 1st week after an AMI have been subjected to the confounding effect of myocardial stunning²⁶ and its variable resolution, which can dwarf the improvement in ejection fraction that is attributed to cell therapy. Because cell delivery in LateTIME will occur weeks after AMI, the effects of stunning should be minimized, so that the true effect of cell therapy can be determined.

Very few clinical studies have been performed to evaluate the efficacy of delayed delivery of stem cells after AMI. Assmus and colleagues²⁷ compared with placebo the administration of intracoronary BMMNCs or cultured circulating blood progenitor cells (CPCs) in 75 patients at least 3 months after MI (mean, 81 ± 72 mo). They noted a small but statistically significant (2.9%) improvement in LVEF by left ventriculography in the BMMNC group (n=35), versus the CPC (−0.4%) or placebo group (−1.2%). In an expanded follow-up to that study,²⁸ Assmus and colleagues administered 214 ± 98 × 10⁶ BMMNCs to 121 patients at a mean of 7 years (range, 4 mo–39 yr) after AMI. They observed an increase in LVEF from 0.399 to 0.417 (*P* < 0.001) by left ventriculography 3 months later. This was accompanied by significant reduction in N-terminal pro-ANP and -BNP levels, and those patients whose cells exhibited the highest levels of colony-forming units and migratory capacity demonstrated a survival benefit at follow-up.

The MYSTAR Trial²⁹ randomized 60 AMI patients with moderate-to-large AMIs (LVEF, <0.45) to a combination of intramyocardial and intracoronary delivery of BMMNCs administered at an early (3–6 wk) or late (3–4 mo) time-point. Compared with baseline values, they observed a 3.5% increase in LVEF 3 months after cell therapy in both groups, as measured by single-photon-emission computed tomography. These findings indicate that the delayed administration of BMMNCs after AMI is safe and feasible and may result in clinical benefit in certain patients. The LateTIME trial will be the 1st protocol to examine the effects of cell delivery at a delayed, but focused, time-point of 2 to 3 weeks after AMI.

Conclusions

LateTIME is consistent with the goal of the CCTRN to accelerate research in the use of cell-based therapies for the management of cardiovascular diseases. Late-

TIME will provide data on a stage of clinical development—the subacute time-frame after AMI—that has not been much studied. This will be the 1st randomized trial in which cells are delivered 2 to 3 weeks after AMI, a time-frame bracketed by the acute and chronic phases of AMI, both of which already have growing safety profiles in regard to BMMNC delivery. When the results become available, this study should provide insight into the clinical feasibility and appropriate timing of autologous cell therapy in high-risk patients after AMI and PCI.

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