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Cell Therapy Following Acute Myocardial Infarction: Do Recent Clinical Trial Results Still Warrant Enthusiasm?

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With advancements in pharmacologic and mechanical reperfusion strategies, survival following myocardial infarction (MI) has greatly improved. Strategies to shorten the time from symptom-onset to treatment in MI have been the focus of considerable study. However, even when revascularization therapy is initiated expeditiously, many patients fail to have significant recovery of cardiac function. Therefore, a number of preclinical and clinical studies have examined the use of cell therapy to restore cardiac function post-MI. Preclinical studies suggest that various types of circulating or bone marrow-derived cells can reduce infarct size, stimulate angiogenesis, attenuate remodeling, and improve myocardial contractility following MI.[1] Based on these encouraging results, clinical trials have been initiated to assess the safety and efficacy of these approaches in humans. In a previous issue of *Cardiology Rounds*, the early safety trials of cell therapy were discussed. In this issue, we focus on recent efficacy trials involving intracoronary catheter-based cell delivery or bone marrow (BM) cell mobilization with granulocyte colony-stimulating factor (G-CSF).

Mechanisms of cell therapy: lessons from pre-clinical studies

Myocardial regeneration

One suggested mechanism of benefit of cell therapy following MI is regeneration of myocardium in the infarct zone. This theory is based on the view that stem or progenitor cells transdifferentiate into, or fuse with, mature cardiomyocytes, and physically regenerate the damaged myocardium. In 2001, Orlic et al first reported regeneration of myocardium by hematopoietic stem cells (HSCs) injected into the infarct border zone in a mouse MI model.[2] However, transdifferentiation of HSCs into myocytes has been strongly refuted by several studies that show maintenance of hematopoietic characteristics of transplanted cells despite localizing to the peri-infarct regions.[3,4] Supporting the view that transplanted cells can regenerate the myocardium, several cell types have been shown to differentiate into cardiomyocytes, including endothelial progenitor cells (EPCs),[5,6] $CD34^+$-enriched stem cells,[7] and BM stromal cells (or mesenchymal stem cells [MSCs]).[8-10] With many contrasting pre-clinical studies, there is no consensus as to the extent of cardiomyocyte replacement that occurs following cell delivery.

Myocardial neovascularization

Administration of circulating or BM-derived cells stimulates myocardial neovascularization, which can improve cardiac function. In small animal models of myocardial ischemia or infarction, systemically-administered or BM-recruited EPCs stimulate angiogenesis and arteriogenesis, with subsequent improvement in myocardial perfusion.[11,12] Similarly, in a pig model of myocardial ischemia, Kawamoto et al[13] reported that autologous EPCs increased capillary density and collateral development (by angiography) and was associated with improvement in left ventricular ejection fraction (LVEF). Studies using BM-derived mononuclear cells (BM-MNCs or bone marrow cells, BMCs) have shown results similar to those obtained with peripheral blood EPCs. In a rat model of cardiac ischemia, BMC implantation induced angiogenesis and improved perfusion in ischemic myocardium.[14] In addition, BMC delivery leads to improved collateral flow,[15,16] augmented capillary density, and reduced contrast echocardiography perfusion defects[17,18] in pigs.

Cell incorporation vs. paracrine stimulation

Although experimental evidence suggests the possibility that cell differentiation and incorporation may improve myocardial function, there are no data demonstrating a definitive correlation between functional improvement and the degree of cell incorporation. In studies of neovascularization, BM transplantation of genetically-modified cells (with reporter genes) have led to varying incorporation rates of BM-derived cells co-expressing endothelial markers (0-90%).[19-23] Some of this variation may be due to different levels of tissue injury in the models of ischemia, types and source of cells, as well as the method

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Table 1: Results from the trials of Janssens et al[40]

LV volume and mass indices, global and regional LV function and late contrast enhancement

Parameter	Difference: 4 days to 4 months		Treatment effect	p
	Control (n=30)	*BMSC (n=30)*		
LVEDV (mL/m²)	2.8	2.8	0.997 (0.915 to 1.086)	0.95
LVESV (mL/m²)	0.6	-1.1	0.980 (0.861 to 1.115)	0.76
Global LVEF (%)	2.2	3.4	1.036 (0.961 to 1.118)	0.36
LV mass index (g/m²)	-5.8	-6.1	0.931 (0.864 to 1.003)	0.06
Late contrast enhancement (g)	-7.9	-10.2	0.717 (0.530 to 0.971)	0.036
Systolic wall thickening in infarct area (%)	1.9	5.7	4.99 (-5.3 to 15.3)	0.35
Systolic wall thickening in border zone (%)	5.7	4.2	-0.84 (-10.5 to 8.9)	0.87

of delivery. Since the incorporation rate of EPCs is often quite low despite positive functional improvement, it has been suggested that neovascularization and myocardial recovery following cell therapy may be due to paracrine effects.[11,24] This is supported by studies showing that these cells have the ability to secrete growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and nitric oxide (NO),[25-27] that could influence the classical process of angiogenesis or prevent cardiomyocyte apoptosis in the peri-infarct zone.[28] In a mouse model of MI, Hiasa et al[29] demonstrated that BM-MNC administration reduced infarct size through secretion of VEGF, which was associated with inhibition of myocyte apoptosis in the peri-infarct zone.[29] It was also recently shown that EPCs stimulate mature endothelial cells and cardiac resident progenitor cells via VEGF, stromal cell-derived factor 1 (SDF-1), and IGF-1 that may enhance angiogenesis and myogenesis *in vivo*.[30] Further studies of the paracrine effects of circulating or BM-derived cells are needed to help elucidate the mechanism of cell therapy.

Despite the lack of concrete mechanistic evidence, positive functional results from several pre-clinical studies have encouraged researchers to use circulating or BM-derived cells in the clinical setting, attempting to regenerate myocardium and/or improve cardiac function following acute MI.

Early trials employing cell therapy for acute MI

In 2002, studies in Europe and North America began enrolling patients into clinical trials examining the administration of BMCs, in conjunction with optimal conventional treatment. These patients underwent primary angioplasty with stent implantation to restore antegrade perfusion of the myocardium at risk, followed by infusion of cells into the infarct-related coronary artery. Most studies used unselected BM-MNCs that included stromal cells, vascular cells, adipocytes, osteoblasts, osteoclasts, MSCs, and HSCs, and only a few reports used selected cell populations (ie, $CD133^+$). Results from >100 patients in various trials suggest that intracoronary delivery of unselected BMCs is safe when given within several months of MI.[31-36] One trial, TOPCARE-AMI, also involved administration of EPCs derived from peripheral blood and found similar results.[34] Cell infusions into the culprit artery after stenting did not appear to result in further damage to the myocardium, nor did this lead to a systemic inflammatory reaction, based on analysis of serum troponins and C-reactive protein (CRP) levels. BMC and EPC transfer did not increase the rate of ventricular or supraventricular arrhythmias, as assessed by Holter monitoring and clinical surveillance[31,35] and, in most patients, in-stent restenosis was not increased due to cell transfer.[32,34,36]

Table 2: Summarized 6-month follow-up results from the ASTAMI trial[41]

Modality	Measure	Stem cells	Control	p
SPECT	Change in EF (%)	8.1	7.0	0.63
	Change in EDV (mL)	-11.2	-1.8	0.11
	Change in infarct size (%)	-11.0	-7.8	0.14
MRI	Change in EF (%)	1.2	4.3	0.05
	Change in EDV (mL)	-6.9	-2.7	0.50
	Change in infarct size (%)	-0.7	-2.6	0.09
Echo	Change in EF (%)	3.1	2.1	0.54
	Change in EDV (mL)	8.9	10.8	0.74

SPECT = single photon emission computed tomography, MRI = magnetic resonance imaging

The early trials were not designed to assess efficacy and, with the exception of the BOOST trial, did not include a randomized control group. For ethical reasons, even the BOOST trial did not incorporate sham procedures, thus limiting the ability to blind patients and investigators. With these caveats in mind, most trials suggest that intracoronary injection of unselected BMCs or EPCs enhances regional wall motion within the infarct area.[31,32,35-38] In both TOPCARE-AMI and BOOST, regional wall motion was associated with a significantly improved global LVEF.[31,36] In the BOOST trial, there was a 6% increase in LVEF at 6-month follow-up in patients receiving cell therapy compared to controls.[36] However, after 18 months, there was no significant difference in LVEF between patients receiving placebo and cell therapy, mainly due to an improvement in control patients.[39] Despite not being powered to analyze efficacy, these trials showed promising results and confirmed the need for placebo-controlled, double-blinded trials.

Recent trials

In the past 2 years, several larger, Phase II trials have added efficacy data to established safety trials' results. Janssens et al[40] published the results of a randomized, double-blind, placebo-controlled study conducted in Belgium. The investigators harvested bone marrow 1 day after successful percutaneous coronary intervention (PCI) for ST-segment elevation MI (STEMI) and assigned patients with optimum medical treatment to infusion of placebo (n=34) or BMCs into the infarct-related artery. Their endpoints included the increase in LVEF (1st endpoint), as well as change in infarct size and regional LV function at 4 months' follow-up (all by magnetic resonance imaging [MRI]). They also used serial ^{11}C-acetate positron emission tomography (PET) to assess any changes in myocardial perfusion and oxidative metabolism. Table 1 shows the full results after a 4-month follow-up. The results were mixed. The authors did not observe a significant difference in mean global LVEF, but reported that BMC transfer was associated with a significant reduction in infarct size (BMC treatment effect -28%) and a better recovery of regional systolic function. Myocardial perfusion and metabolism increased similarly in both groups. There were no complications associated with BMC transfer. This trial confirmed the safety of this approach, but did not achieve its primary endpoint, of improving LVEF. The improvements in infarct size and systolic function confirm, however, that this approach may still be feasible for patients suffering from acute MI.

Cell therapy was a major topic at the 2005 Scientific Sessions of the American Heart Association (AHA) in Dallas, Texas. In addition to several trials for the treatment of heart failure, peripheral artery disease, and angina pectoris, results of 2 other trials using BMCs for STEMI were presented.

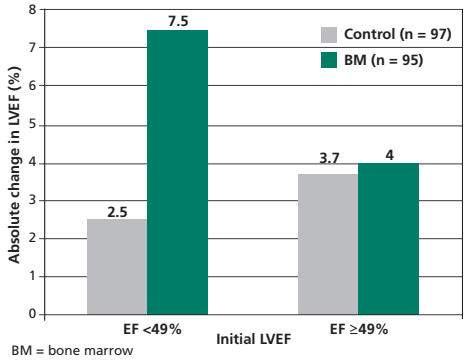
Table 3: REPAIR-AMI summarized results: 4-month follow-up[42]

	Control (n = 92)	BM (n = 95)	p
EF at baseline (%)	47 ± 1	48 ± 1.5	0.310
EF at 4 months (%)	50 ± 1.5	54 ± 1.1	0.021
Absolute change in EF (%)	3.0 ± 0.7	5.5 ± 0.7	0.014

The ASTAMI study: Dr. Ketil Lunde, from Oslo, Norway, presented results from the Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI) study, a phase II, randomized, controlled, prospective study[41] that enrolled 101 patients with acute anterior wall STEMI treated with acute PCI. They were then randomized 1:1 to either intracoronary transplantation of autologous BM-MNCs (n = 52) or to placebo (n = 49) 4-8 days after the MI. This study was not blinded since the control group did not have a BM aspirate or repeat catheterization. The primary outcome of the study was improvement in LVEF assessed by electrocardiogram (ECG)-gated single photon emission computed tomography (SPECT) (MRI and echo were also used). Secondary outcomes included exercise capacity and quality of life. The investigators limited enrolment to patients with anterior wall involvement since it is the region of the myocardium best visualized with noninvasive imaging and is associated with a greater risk of post-MI LV dysfunction compared with isolated involvement of other walls. Table 2 summarizes the data from this trial. Results with 3 different imaging modalities revealed no differences in LVEF between groups at baseline and at 6 months' follow-up. There were also no differences in change in infarct size and end-diastolic volume, no deaths at 6 months, and adverse event rates did not differ between groups. Although this trial did not show any benefit of cell therapy, Dr. Lunde stated that "results in this area of study remain inconclusive and that further research is needed to explore new methods for cell therapy in acute MI."[41]

The REPAIR-AMI trial: In the same session at the AHA, Dr. Andreas Zeiher from Frankfurt, Germany, presented results of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial.[42] This was a randomized, double-blind, placebo-controlled, multicentre (17 centres in Germany and Switzerland) trial using BM-MNCs in AMI patients who had undergone successful reperfusion therapy. Between the 3rd and 5th day following infarction, 204 patients underwent BM aspiration and were randomized to receive intracoronary infusion of either BMCs (n=101) or placebo (n=103). In contrast to ASTAMI, all patients received BM aspirate to ensure proper blinding. After 4 months, all patients underwent follow-up left ventriculography. An increase in LVEF was observed in both groups, but the increase was significantly higher in patients randomized to the BMC infusion, although the actual difference was marginal (2.5%) (Table 3). There were more pronounced differences when analyzing subgroups. The benefits of BMC therapy were greater in patients with more severe LV dysfunction (EF <49%); in this subset of patients, the absolute change in EF in the BMC arm was 7.5% vs only 2.5% in the placebo arm (Figure 1). In addition, delaying time to infusion was associated with better outcomes. Patients in the BMC arm treated after day 5 had the largest increment in EF (Figure 2), while there was no difference in the absolute change in LVEF in patients who received treatment ≤4 days after MI. The results of REPAIR-AMI suggest that intracoronary infusion of BM-MNCs in patients with reperfused AMI is associated with improved global LV contractile function and preferentially improves LV function in patients with the most severely depressed contractility after AMI and when administered >5 days after the MI.

Figure 1: REPAIR-AMI – Improvement in LVEF according to initial LVEF[42]

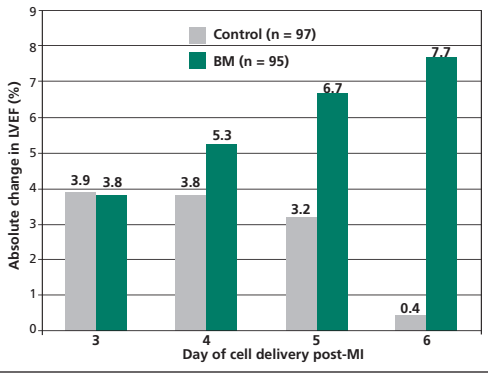


Cell mobilization

An alternative strategy for "cell therapy" involves the mobilization of cells from the BM, thus avoiding the need for cell isolation. In mice, granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) increase BM cell mobilization and stimulate myogenesis and angiogenesis, with an improvement in cardiac function after acute MI.[43,44] Apart from EPC recruitment, G-CSF has been shown to have direct myocardial effects contributing to improved myocardial function, including inhibition of cardiomyocyte and endothelial apoptosis.[45] G-CSF can also accelerate infarct healing by enhancing macrophage infiltration and matrix metalloproteinase activation.[46] If effective, administration of G-CSF could eliminate the need for BM aspiration and interventional administration, making it a very attractive approach. Despite these potential advantages, G-CSF mobilizes inflammatory cells in addition to progenitor cells, which could aggravate chronic inflammation of atherosclerosis and lead to plaque rupture or other complications.[47-50] Several clinical trials for STEMI have used this approach, with mixed results. Although results from 2 small safety trials were positive,[51,52] others indicate that the atherogenic risks outweigh the benefits of G-CSF administration.[53,54] Several medium-sized trials were recently completed, with conflicting results.

The ***FIRSTLINE-AMI trial:*** The Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction (**FIRST**LINE-AMI) trial,[55-57] a randomized, nonblinded trial, was conducted in Germany and enrolled 50 patients: 25 received 10 µg/kg G-CSF for 6 days and 25 received standard care alone. G-CSF was started immediately following PCI (mean 89 minutes). Patients were followed-up 35 days later and after 4 months. G-CSF administration led to a 20-fold increase in circu-

Figure 2: REPAIR-AMI – Improvement in LVEF according to time of cell infusion post-MI[42]



lating CD34+ cells at day 6, without significant changes in blood flow, blood viscosity or inflammatory reaction, or any major adverse effects. As early as 35 days after administration, the G-CSF group had an improved LVEF and no evidence of LV end-diastolic remodelling, which persisted until 4 months (LVEF of 54 ± 8% versus 48±4% at baseline; diameter of 55 ± 5 mm and improved segmental wall thickening). Control patients, however, had a mean LVEF of only 43% ± 5% at 4 months, with increased left ventricular end-diastolic dimension (LVEDD) of 58 ± 4 mm, and no segmental wall thickening. These improvements were corroborated by enhanced metabolic activity and ^{18}F-flurodeoxyglycose (FDG) uptake in the infarct zone (58.9% ± 9% vs. 44% ± 13% uptake in the control group). Although these results appear positive, results from double-blind studies are needed to better elucidate the efficacy of this approach.

The ***REVIVAL-2 trial:*** The results of another German trial, Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells (REVIVAL-2), were recently published.[58] In contrast to FIRSTLINE-AMI, this was a randomized, double-blind, placebo-controlled trial that enrolled 114 patients who received either 10 µg/kg G-CSF (n=56) or placebo (n=58) for 5 days post-AMI. Patients were randomized 5 days after STEMI (which differs from the design of other G-CSF trials, in which treatment started much sooner after MI). The primary endpoint was infarct size, as assessed by SPECT (Tc99m). Secondary endpoints were EF and restenosis incidence. After 4-6 months, there were no differences between the G-CSF and control groups in any of the measured endpoints (infarct size, EDV or ESV, EF or restenosis rate) and concluded that G-CSF had no effect on these parameters of heart function.

The American RECOVER[59] trial was a double-blind, randomized, placebo-controlled trial that administered G-CSF in a dose-escalating manner. Only 18 patients were enrolled in a 2:1 randomization to control (6 patients in each group), with 2-dose phases (to 5 µg/kg for 5 days or to 10 µg/kg for 5 days). Enrollment was limited to patients with large MIs (20%-39% EF) and G-CSF was administered within 48 hours of symptom onset (mean 40 hours). Patients were followed up at 30 days and 12 months. As expected, G-CSF administration led to a 6- to 7-fold increase in CD34+ and CD117+ stem/progenitor cells. However, after 30 days, the LVEF (1st endpoint) was greater in the placebo group than in the G-CSF group, indicating that G-CSF did not improve ventricular function.

The ***STEMMI trial:*** The results of the STEMMI trial were recently published.[60] In this study, 78 STEMI patients were randomized to G-CSF (10 µg/kg) or placebo for 6 days, initiated 1-2 days after MI. The primary endpoint was a change in systolic wall thickening, as assessed by MRI. After 6 months, both the G-CSF and control groups had a 17% improvement in wall thickening, but similar results were not observed in the infarct and noninfarct zones. However, LVEF improved in both groups, as measured by MRI and echocardiography. Finally, there was no difference in adverse events in the 2 groups. The authors concluded that there was no improvement in myocardial function due to G-CSF mobilization, which agrees with most of the recent trials.

Interpretation and comparison of recent trials

Recent results have diffused some of the optimism generated by earlier, primarily safety trials employing cell delivery. Of the 3 large trials using intracoronary cell delivery, only REPAIR-AMI achieved its primary endpoint, with only a mild increase using a relatively insensitive modality, LV angiography. The Janssens trial saw only improvements in infarct size and systolic function, their secondary endpoints. The ASTAMI trial failed to meet its primary endpoint, as assessed using 3 separate imaging modalities. Despite these disappointing results, it would be unwise to rule-out this approach. As Dr. Zeiher pointed out, not all patients may benefit from these therapies and it is critical to tailor future trials around those who satisfy the characteristics associated with the greatest improvements, eg, future studies should be designed to administer cells at least 5 days following the MI and in patients with LVEFs <49%.

In addition, the viability and function of the cells are crucial to the success of these therapies and thus, how the cells are treated prior to administration is important. In this respect, the trials vary greatly. The ASTAMI investigators harvested BMCs and, following the isolation of MNCs, stored the cells in saline overnight. Saline is not buffered and, thus, is not an optimal solution for live cells. In contrast, cells were suspended in cell culture medium in the REPAIR-AMI trial, a medium designed to provide a nutrient-rich, buffered, and homeosmotic environment for the cells.

At the 2nd International Conference on Cell Therapy for Cardiovascular Disease (New York City, January 2006), Dr. Zeiher presented results of experiments comparing these 2 methods of storage. Using a standard *in vitro* assay, he demonstrated that cells stored overnight in saline had <50% of the migratory ability – a surrogate of the regenerative properties – of cells treated according to the methods in the REPAIR-AMI trial.[61] In addition, in patients receiving BMCs in REPAIR-AMI, the migratory ability of their cells *in vitro* strongly correlated with the improvements they gained in the trial. Thus, cell viability and function is critical to a successful trial.

The regenerative capabilities of BM or circulating cells are also related to the age and health of the individual donor and, in the case of autologous therapies, the recipients of cell therapy. Reports have shown that patients with coronary artery disease (CAD) and/or various risk factors for CAD (in particular, diabetes, smoking, hypertension, and hypercholesterolemia) have reduced numbers and function of circulating EPCs,[62-67] correlating with the number of risk factors these patients have.[66] The dysfunction is not restricted to circulating cells; BM-MNCs harvested from patients with ischemic cardiomyopathy also have a profoundly reduced potential for neovascularization.[62] Many patients with heart disease who could benefit from cell therapy have several CAD risk factors that may impair the function of their EPCs and limit the potential benefit of such therapies. Improving the regenerative properties of the cells prior to administration may be a favourable approach to improving the effectiveness of these therapies.

Dimmeler et al (Frankurt) recently presented the preliminary results from studies using a small molecule that enhances the expression of endothelial nitric oxide synthase (eNOS), one of the proteins required for the production of nitric oxide (NO). Endothelial dysfunction is considered to be primarily a reduced bioavailability of NO and, as such, increasing the production of NO has been shown to reverse this dysfunction.[68] Dr. Dimmeler has

previously shown that eNOS is critical to the proper function of EPCs[69] and, recently, presented data showing that patients with CAD have reduced expression of eNOS.[70] Preliminary unpublished data using the eNOS enhancer (AVE-9488) indicate that it increases eNOS expression roughly 2-fold in EPCs[70] and this is sufficient to reverse the dysfunction of EPCs taken from patients with ischemic cardiomyopathy.[70] Although applying this agent in this way has not yet been tested in clinical trials, these results are encouraging and indicate that modifying cells, either pharmacologically or genetically, may help improve their regenerative ability and potentially increase the efficacy of clinical trials using autologous administration of cells.

Like the trials employing cell delivery, G-CSF trials are revealing more negative than positive results. In addition, use of G-CSF has potential safety concerns, making it a contentious agent for clinical use in this patient population. Although the STEMI trials with G-CSF have not shown any negative safety results, Hill et al demonstrated that in a trial for refractory angina, G-CSF led to an increase in the number of ischemic events and a rise in serum CRP. The trial was stopped prematurely due to adverse events.[71] As with the cell delivery trials, the contrasting results between the trials have not yet been explained but, presumably, differences in timing and duration of G-CSF treatment are responsible for some of the differences. In addition, the effect of CAD risk factors on cell function is still relevant since mobilization is only as effective as the cells themselves are functional. Since the cells are never extracted, *ex vivo* cellular manipulations are impossible, leaving only systemic pharmacological therapy as a means of improving cell function. While larger trials are warranted to further investigate this approach, they should still be designed with safety as an utmost priority.

Future directions

The recent publication and presentation of randomized and blinded trials of cell therapy for acute STEMI have dampened the excitement generated by early positive results. The mixed results from very similar trials indicate that cell therapy is not the panacea that many had hoped for following the disappointing results of myocardial gene therapy. However, these trials do provide important insights. The REPAIR-AMI study revealed that BM-MNC administration does not necessarily work for all STEMI patients, rather those with more severe LV dysfunction. What has also become clear is the importance of cell function prior to administration. There is no consensus on the most appropriate way to isolate, handle, and prepare the cells for delivery, or which type of cell is the most beneficial. Since the trials to date have used different methods, results are difficult to directly compare. In the future, it will be important to validate a trial's standard operating procedures according to cell function parameters, including perhaps viability, migratory ability, and angiogenic capacity. In the same way, improving the regenerative capacity of cells obtained from diseased individuals may help generate more positive results. Dramatic differences between cells obtained from young, healthy individuals and those obtained from elderly CAD patients[62,72] may explain the marginal results found in trials of both autologous cell delivery and G-CSF stimulated cell mobilization. Pharmacologic or genetic *ex vivo* manipulation of circulating or BM- derived cells will likely be incorporated into new trials following preclinical validation.

The study of cell therapy for myocardial injury is still in its infancy and there is is still much to be learned from preclinical and clinical studies. It is critical for investigators to proceed with prudence, perform properly designed randomized, controlled trials founded on the lessons learned thus far. It would be unfortunate for a field with such potential to be discredited on the basis of results obtained from poorly-designed clinical trials.

References

1. Wollert KC, Drexler H. Clinical applications of stem cells for the heart. *Circ Res* 2005;96:151-163.
2. Orlic D, et al. Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann NY Acad Sci.* 2001;938:221-229.
3. Balsam LB, et al. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 2004;428:668-673.
4. Murry CE, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004;428:664-668.
5. Badorff C, et al. Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. *Circulation* 2003;107:1024-1032.
6. Rupp S, et al. Statin therapy in patients with coronary artery disease improves the impaired endothelial progenitor cell differentiation into cardiomyogenic cells. *Basic Res Cardiol* 2004;99:61-68.
7. Yeh ET, et al. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 2003;108:2070-2073.
8. Makino S, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 1999;103:697-705.
9. Mangi AA, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 2003;9:1195-1201.
10. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002;105:93-98.
11. Kawamoto A, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001;103:634-637.
12. Asahara T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999;85:221-228.
13. Kawamoto A, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation* 2003;107:461-468.
14. Nishida M, et al. Improvement of cardiac function by bone marrow cell implantation in a rat hypoperfusion heart model. *Ann Thorac Surg* 2003;75:768-773.
15. Fuchs S, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol* 2001;37:1726-1732.
16. Vicario J, et al. Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. *Cardiovasc Radiat Med* 2002;3:91-94.
17. Hamano K, et al. Therapeutic angiogenesis induced by local autologous bone marrow cell implantation. *Ann Thorac Surg* 2002;73:1210-1215.
18. Kamihata H, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001;104:1046-1052.
19. Murayama T, et al. Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization in vivo. *Exp Hematol* 2002;30:967-972.
20. Crosby JR, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. *Circ Res* 2000;87:728-730.
21. Jackson KA, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001;07:1395-1402.
22. Llevadot J, et al. HMG-CoA reductase inhibitor mobilizes bone marrow-derived endothelial progenitor cells. *J Clin Invest* 2001;108:399-405.
23. Lyden D. et al. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* 2001;7:1194-1201.
24. Kocher AA, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7:430-436.
25. Ii M, et al. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity. *Circulation* 2005;111(9):1114-20.
26. Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003;10:1164-1169.
27. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 2004;95:343-353.
28. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995;1:27-31.
29. Hiasa K, et al. Bone marrow mononuclear cell therapy limits myocardial infarct size through vascular endothelial growth factor. *Basic Res Cardiol* 2004;99:165-172.
30. Urbich C, et al. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol* 2005;39:733-742.

31. Assmus B, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 2002; 106:3009-3017.
32. Fernandez-Aviles F, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. *Circ Res* 2004;95:742-748.
33. Kuethe F, et al. Lack of regeneration of myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans with large anterior myocardial infarctions. *Int J Cardiol* 2004;97:123-127.
34. Schachinger V, 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;44:1690-1699.
35. Strauer BE, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106: 1913-1918.
36. Wollert KC, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004;364:141-148.
37. Stamm C, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003;361:45-46.
38. Stamm C, et al. CABG and bone marrow stem cell transplantation after myocardial infarction. *Thorac Cardiovasc Surg* 2004;52:152-158.
39. Meyer GP, Wollert KC, Lotz J, Steffens J, et al. Bone marrow transfer to enhance ST-elevation infarct regeneration: long-term magnetic resonance imaging follow-up data from the BOOST-trial. *Circulation* 2004; 110:(suppl III):239.
40. Janssens S, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet* 2006;367:113-121.
41. Lunde K, et al. ASTAMI: Autologous mononuclear bone marrow cells in acute anterior wall myocardial infarction. late breaking clinical trial. American Heart Association Scientific Sessions, November 2005. Dallax, TX.
42. Zeiher, et al. REPAIR-AMI. Late Breaking Clinical Trial. American Heart Association Scientific Sessions, November 2005. Dallax, TX
43. Ohtsuka M, et al. Cytokine therapy prevents left ventricular remodeling and dysfunction after myocardial infarction through neovascularization. *FASEB J* 2004; 18:851-853.
44. Orlic D, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci US* 2001; A 98:10344-10349.
45. Harada M, et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. *Nat Med* 2005;11:305-311.
46. Minatoguchi S, et al. Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. *Circulation* 2004;109:2572-2580.
47. Conti JA, Scher HI. Acute arterial thrombosis after escalated-dose methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy with recombinant granulocyte colony-stimulating factor. A possible new recombinant granulocyte colony-stimulating factor toxicity. *Cancer* 1992;70:2699-2702.
48. Fukumoto Y, et al. Angina pectoris occurring during granulocyte colony-stimulating factor-combined preparatory regimen for autologous peripheral blood stem cell transplantation in a patient with acute myelogenous leukaemia. *Br J Haematol* 1997;97: 666-668.
49. Hill JM, et al. Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. *J Am Coll Cardiol* 2005;46:1643-1648.
50. Vij R, et al. Unstable angina in a peripheral blood stem and progenitor cell donor given granulocyte-colony-stimulating factor. *Transfusion* 1999;39:542-543.
51. Kang HJ, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet* 2004;363:751-756.
52. Valgimigli M, et al. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. *Eur Heart J* 2005;26:1838-1845.
53. Hill JM, et al. Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. *J Am Coll Cardiol* 2005;46:1643-1648.
54. Vij R, et al. Unstable angina in a peripheral blood stem and progenitor cell donor given granulocyte-colony-stimulating factor. *Transfusion* 1999;39:542-543.
55. Nienaber CA, et al. Effects of granulocyte-colony-stimulating factor on mobilization of bone-marrow-derived stem cells after myocardial infarction in humans. *Nat Clin Pract Cardiovasc Med* 2006; 3(Suppl 1):S73-S77.
56. Ince H, et al. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). *Circulation* 2005;112:3097-3106.
57. Ince H. et al. Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction: final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) Trial. *Circulation* 2005;112: I73-I80.
58. Zohlnhofer D, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. *JAMA* 2006;295:1003-1010.
59. Ellis SG. RECOVER: First US study of GCSF in AMI. Program and abstracts from the Second International Conference on Cell Therapy for Cardiovascular Diseases; January 19-21, 2006; New York, NY.
60. Ripa RS, et al. Stem cell mobilization induced by subcutaneous granulocyte-colony stimulating factor to improve cardiac regeneration after acute ST-elevation myocardial infarction: result of the double-blind, randomized, placebo-controlled stem cells in myocardial infarction (STEMMI) trial. *Circulation* 2006; 113:1983-1992.
61. Zeiher A. Oral presentation. 2nd International Conference on Cell Therapy for Cardiovascular Disease; New York City, January 2006.
62. Heeschen C, et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 2004;109:1615-1622.
63. Verma S, et al. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. *Circulation* 2004;109:2058-2067.
64. Heiss C, et al. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 2005;45:1441-1448.
65. Urbich C, Dimmeler S. Risk factors for coronary artery disease, circulating endothelial progenitor cells, and the role of HMG-CoA reductase inhibitors. *Kidney Int* 2005;67:1672-1676.
66. Vasa M, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89: E1-E7.
67. Tepper OM, et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002;106:2781-2786.
68. Cai S, Khoo J, Channon KM. Augmented BH4 by gene transfer restores nitric oxide synthase function in hyperglycemic human endothelial cells. *Cardiovasc Res* 2005;65:823-831.
69. Aicher A, et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med* 2003; 9:1370-1376.
70. Dimmeler S. Oral presentation. 2nd International Conference on Cell Therapy for Cardiovascular Disease (New York City, January 2006)
71. Hill JM, et al. Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. *J Am Coll Cardiol* 2005;46:1643-1648.
72. Heiss C, et al. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 2005;45:1441-1448.

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