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Cell-Based Cardiac Repair Reflections at the 10-Year Point

Charles E. Murry, MD, PhD; Loren J. Field, PhD; Philippe Menasché, MD, PhD

It has now been more than a decade since the first experiments were performed using cell transplantation for the prevention and treatment of heart failure.¹⁻³ Although the biomedical community was initially somewhat skeptical of this approach, a large body of experimental evidence was amassed showing that injected cells could create new tissue and improve function of the failing heart. This evidence, coupled with the recognized limitations of heart failure treatments and the intuitively appealing concept of “regenerative medicine,” has contributed to a crescendo of activity in cell-based cardiac repair. Given the flurry of clinical trials that are currently under way, we think it is timely to review progress over the past 10 years and provide a critical assessment of where the field stands and where it appears to be headed.

The Early Years: Transplantation of Committed Cells in Preclinical Studies

Cell-based cardiac repair began with studies of skeletal myoblasts derived from skeletal muscle satellite cells.¹⁻³ Myoblasts were the initial choice because of their availability from autologous or syngeneic sources, their ability to proliferate, and their ability to withstand ischemia better than many cell types. Although it was originally hoped that these cells would transdifferentiate into cardiomyocytes, it is now clear that myoblasts remain stubbornly committed to form mature skeletal muscle in the heart³⁻⁵ (with the exception of rare cell fusion events at the graft–host interface⁶). Skeletal muscle is one of the few cell types in the body that does not normally express gap junction proteins, and hence, structural and physiological studies indicate that skeletal muscle cells do not form electromechanical junctions with cardiomyocytes when engrafted into the heart.^{7,8} Despite this, numerous studies have shown beneficial effects of skeletal myoblast grafting into the infarcted heart in rodents and large animals.⁸⁻¹³

Cardiomyocytes would seem the optimal cell type to repair an infarct, and attention quite logically turned to this cell type next. Field’s group showed that fetal cardiomyocytes could form stable grafts in uninjured hearts of syngeneic recipients.¹⁴ Subsequent studies from multiple investigators

showed that fetal or neonatal cardiomyocytes could form new myocardium in injured hearts as well.¹⁵⁻¹⁸ The initial enthusiasm generated by these studies cooled somewhat, however, when it was shown that massive cell death, coupled with only limited cell proliferation after transplantation, prevented formation of enough new myocardium to replace more than a tiny fraction of an infarct.^{19,20} Still, functional improvement was reported even with small grafts,^{18,21-23} prompting the search for a cardiac cell source for human applications.

Other committed cell types such as fibroblasts and smooth muscle cells, which clearly cannot contract like cardiomyocytes, also were reported to enhance function of the injured heart.^{24,25} These studies were informative, because they suggested that noncontractile effects might be at play in cell-based repair. An emerging hypothesis has been called the “paracrine effect,” whereby transplanted cells are proposed to produce growth factors, cytokines, and other local signaling molecules that are beneficial to the infarct. Although little is known right now, possible mechanisms include increasing perfusion through angiogenesis and arteriogenesis, improvements in the infarct connective tissue such that less ventricular dilation occurs, and enhancement of myocyte or other cell survival. Multiple investigations are now under way to identify possible paracrine signaling pathways that could contribute to enhanced cardiac performance.

Stem Cells in Preclinical Studies of Cardiac Repair

The latter part of the 1990s heralded several important breakthroughs in stem cell biology, and with them, hopes for true regenerative healing of the heart were raised. In 1996, Ian Wilmut’s group cloned Dolly by fusing a sheep’s udder cell with an enucleated sheep oocyte.²⁶ The ability to derive an entire animal from a single somatic cell taught us that the differentiation state of an adult nucleus was reversible and could be reprogrammed to generate any other cell type. This breakthrough changed our thinking in several important ways. The first was the possibility that nuclear transfer could be used to reprogram nuclei from patients, in effect creating “designer” stem cells with genetic identity to the patient

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(excepting mitochondrially encoded genes). The second was the notion that adult cells, and adult stem cells in particular, might have a greater ability to differentiate into other cell types than originally envisioned (see below).

Embryonic stem cells emerged as promising candidates for cardiac repair in the late 1990s. Although it had been known for some time that these cells could give rise to cardiomyocytes,²⁷ there were several difficulties that slowed their use for cardiac repair applications. This includes the increased technical difficulty of growing these cells and keeping them undifferentiated, the low efficiency with which cardiac differentiation occurs spontaneously, and the challenge of purifying cardiomyocytes from the many other cell types that form during spontaneous differentiation. In 1996, Field's group reported a genetic technique for selecting highly purified cardiomyocytes, using the cardiac-specific α -myosin heavy chain promoter to drive expression of an antibiotic resistance gene.²⁸ They showed that cardiomyocytes derived by this approach formed stable grafts in uninjured mouse hearts without tumor formation. In 1998, the long-sought isolation of human embryonic stem cells was achieved by Jamie Thomson's laboratory at the University of Wisconsin.²⁹ The subsequent generation of human cardiomyocytes from human embryonic stem cells by the Gepstein³⁰ and Carpenter³¹ groups provided a rational source for cardiomyocytes in eventual allogeneic clinical trials. Transplantation work with human cardiomyocytes is just getting under way and will be described below.

Perhaps the most surprising reports, however, came from adult stem cells. Long recognized for their ability to repopulate labile tissues such as blood and epithelium, stem cells were discovered in classically stable tissues such as the brain,³² and recent evidence suggests the presence of stem cells in myocardium as well (discussed below). Furthermore, multiple studies suggested the possibility that adult stem cells could "transdifferentiate," or acquire an unexpected phenotype when placed in new environments, such as an injured tissue. For several years, the literature was replete with reports such as marrow \rightarrow endothelium,^{33,34} marrow \rightarrow brain/neuron,³⁴⁻³⁶ brain \rightarrow blood,³⁷ marrow \rightarrow skeletal muscle,^{38,39} skeletal muscle \rightarrow blood,⁴⁰ marrow \rightarrow epithelium,^{34,41,42} brain \rightarrow whole embryo,⁴³ and, of particular interest for this article, marrow \rightarrow cardiomyocytes.⁴⁴⁻⁴⁶ This abundance of reports led to the notion that adult stem cells were actually highly plastic and that the traditional developmental boundaries, such as endoderm, mesoderm, and ectoderm, could be readily crossed. More important for clinical medicine, these reports also raised hopes that dramatic cures for diseases of cell deficiency, such as myocardial infarction, were just around the corner.

After a few years, however, the pendulum began to swing in the other direction, as alternate techniques were used to follow the fates of adult stem cells. For example, the apparent conversion of skeletal muscle \rightarrow blood actually resulted from the unexpected presence of hematopoietic stem cells in solid tissues, rather than transdifferentiation of true muscle stem cells.⁴⁷ Transdifferentiation of neural stem cells \rightarrow blood could not be replicated in more detailed studies from another laboratory.⁴⁸ Conversion of bone marrow \rightarrow liver was shown

to result entirely from fusion of macrophages with host hepatocytes and subsequent reprogramming of the macrophage nucleus, rather than true transdifferentiation.^{49,50} Considering cardiovascular research more specifically, generation of endothelial cells from bone marrow progenitors has been reproduced in multiple laboratories and therefore seems to be a robust finding.^{44,51} Conversely, formation of new cardiomyocytes from hematopoietic stem cells now appears to be limited to extremely rare cell fusion events (eg, 1 in 10 000 cells),⁵² and several groups have failed to replicate the previous report of widespread cardiac regeneration after direct injection of hematopoietic stem cells.⁵³⁻⁵⁵ Although the basis for this discrepancy is currently unknown, possible explanations include differences in the rigor of criteria used to define a cardiomyocyte and (although unlikely) subtle differences in the stem cell populations isolated.

Although bone marrow currently appears to be unable to generate significant numbers of cardiomyocytes, several groups have reported evidence that myocardium itself contains a resident progenitor cell population capable of giving rise to new cardiomyocytes. Candidate myocardial progenitor populations include cells expressing the receptor tyrosine kinase c-kit,^{56,57} those expressing stem cell antigen-1 (Sca-1), cells capable of effluxing the DNA-binding dye Hoechst 33258 (so-called side population, or SP, cells),^{58,59} and most recently, cells expressing the transcription factor islet-1.⁶⁰ Surprisingly, there seems to be little overlap among these subsets of myocardial cells, eg, myocardial c-kit cells do not express Sca-1 and vice versa. At the moment, it seems paradoxical that the heart, with its inadequate regenerative capacity, would have multiple subpopulations of cardiac progenitor cells. Nonetheless, the roles of these cells in tissue maintenance, repair, and possible therapeutic applications will be an exciting area of investigation in the coming years.

Skeletal Muscle: The First Clinical Candidate for Human Cardiac Repair

Skeletal myoblasts were the first to enter the clinical arena, after completion of a decade of experimental testing resulting in at least 40 studies (primarily involving animal models of myocardial infarction; reviewed by Dowell et al⁶¹). As discussed above, these studies have consistently shown differentiation of implanted myoblasts into multinucleated myotubes (not cardiomyocytes) with the absence of electromechanical coupling between these engrafted myotubes and host cardiomyocytes. Despite these apparent shortcomings, a definite improvement in regional and global left ventricular function has been found. These data, along with the clinically appealing characteristics of skeletal myoblasts (a high *in vitro* scalability of the initial biopsy, an advanced stage of differentiation virtually eliminating tumorigenicity, and a high resistance to ischemia), have paved the way for the initial human trials, which started in June 2000.⁶² Thus far, 6 phase I safety and feasibility pilot studies of autologous skeletal myoblast transplantation have been performed. Autologous myoblasts were isolated from muscle biopsies by enzymatic dispersion, and the cells were expanded for several weeks in culture by use of fetal bovine serum (tested free from bovine spongiform encephalopathy prion) as a mitogen. All studies

TABLE 1. Skeletal Myoblast Trials in Chronic Ischemic Disease (Surgical Studies)

Study	Cell Dose	Controls	Revascularization of the Transplanted Segments	Result
Menasché et al ⁶³ (n=10)	871×10 ⁶ (86% CD56+)	None	No	<ul style="list-style-type: none"> ● Improved symptoms (NYHA) ● Improved EF (by 8% at 10.9 months) ● Improved regional contractility of 63% of the myoblast-implanted segments (echo) ● 4 early postoperative VT (nonfatal) before systematic implementation of perioperative amiodarone prophylaxis
Herreros et al ⁶⁴ (n=12)	221×10 ⁶ (65.6% CD56+)	None	Yes	<ul style="list-style-type: none"> ● Improved EF (by 18% at 3 months) ● Improved regional contractility of myoblast-implanted segments (echo) ● Improved viability of myoblast-implanted segments (¹⁸F-FDG PET)
Siminiak et al ⁶⁶ (n=10)	4×10 ⁵ to 5×10 ⁷ (65.4% desmin+)	None	Yes	<ul style="list-style-type: none"> ● Improved EF (by 7% at 4 months) ● Improved regional contractility of some myoblast-implanted segments (echo) ● 2 early postoperative VT and 2 VT at 2 weeks (no additional case after systematic implementation of perioperative amiodarone prophylaxis)

Cells were injected in multiple sites across scarred segments during standard coronary artery bypass grafting operations.

NYHA indicates New York Heart Association; EF, ejection fraction; VT, ventricular tachycardia; and ¹⁸F-FDG PET, ¹⁸F-labeled fluoro-deoxyglucose positron emission tomography.

focused on patients with severe left ventricular dysfunction caused by myocardial infarction, and cell injections were targeted to discrete akinetic and metabolically inactive scars. Four of these studies were surgical,^{63–66} ie, entailed myoblast implantation at the time of coronary artery bypass grafting or left ventricular assist device implantation, whereas the remaining 2 were designed as catheter-based stand-alone procedures and used either an endoventricular⁶⁷ or a coronary sinus transvenous⁶⁸ approach. The surgical studies for which full articles are available are summarized in Table 1.

The major findings of these trials can be summarized as follows. (1) The technical feasibility of the procedure is now well established, in that hundreds of millions of myoblasts can be grown from a small muscle biopsy under Good Manufacturing Practice conditions and subsequently re-injected into the target scar without specific procedural complications. (2) Long-term engraftment of myoblasts, featuring clusters of skeletal myofibers aligned parallel to host cardiomyocytes and embedded in scar tissue, has been documented by pathological studies up to 18 months after surgical transplantation.^{65,69} Although histological confirmation of engraftment is encouraging, it should be noted that the skeletal muscle grafts are only a small fraction of the left ventricular mass. Given the large number of cells injected (hundreds of millions), this probably indicates that the majority of cells are lost, either to inefficient seeding (leaking out the injection site or into the systemic circulation) or to death shortly after implantation. Thus, one of the major challenges for cell-based cardiac repair will be the development of strategies to optimize cell retention (eg, through improved injection systems) and enhanced survival (eg, through induction of angiogenesis or prevention of cell death⁷⁰). (3) Safety has been a concern, because of the report

of ventricular tachycardia in 4 of 10 patients in the French trial.⁶³ In this regard, however, it is important to note that the inherently arrhythmogenic substrate of the failing heart requires randomized, controlled trials to determine a causal relationship. (4) No meaningful conclusions can yet be drawn regarding efficacy in restoration of function in the injected areas (for reasons discussed below), but early functional results have indicated some positive trends.

Collectively, these results indicate that phase II skeletal myoblast clinical trials are warranted. Such studies should be designed to overcome the shortcomings that made assessment of functional efficacy in the phase I trials problematic. These include the lack of randomized control groups and the concomitant coronary bypass surgery in many studies (sometimes including the region receiving myoblasts). Furthermore, there are differences among published studies that make direct comparisons difficult, including differences in cell culture processes (which may influence myoblast viability and differentiation), the variable end points used to judge efficacy (stress echocardiography, nuclear angiograms, MRI, positron emission tomography), and the variable baseline function of the engrafted regions (which ranged from hypokinetic to dyskinetic).

To overcome many of these issues, one of us (P.M.) has implemented a large-scale, multicenter, double-blind, placebo-controlled, dose-ranging, randomized study with contractility of the grafted regions as the primary end point. Furthermore, defibrillators will be implanted in all study patients. These devices will provide a safety net and potential survival benefit, as well as assessing the incidence and timing of potential graft-related arrhythmias. So far, the data yielded by the first unblinded safety analysis have been reassuring, and the study has been approved to move forward. We expect that this study will

TABLE 2. Bone Marrow Trials in Chronic Ischemic Disease

Study	BMMC Dose	Controls	Result
Perin et al ⁸⁰ (n=14)	2.5×10 ⁷ (6×10 ⁵ CD34+)	Nonrandomized sequentially enrolled. (n=7) No sham injection.	<ul style="list-style-type: none"> ● Reduced symptoms of CHF (NYHA) ● ESV reduced ● Improved EF (6%) ● Improved MBF ● Trends toward improved EDV, $\dot{V}O_2$, treadmill time ● Improved electromechanical function by NOGA late vs early (no controls for this)
Fuchs et al ⁹⁹ (n=10)	7.9×10 ⁷ (2×10 ⁶ CD34+)	None	<ul style="list-style-type: none"> ● Improved angina score ● Improved coronary flow after adenosine ● No effect on EF or wall motion
Tse et al ⁷⁹ (n=8)	Not reported (40 mL marrow aspirated)	None	<ul style="list-style-type: none"> ● No change in EF ● Improved wall thickening and radial shortening before vs after injection (MRI) ● Modestly reduced hypoperfused zone before vs after injection (contrast MRI)

Cells were delivered by intramyocardial injection via NOGA-guided intraventricular catheter. Cell dose refers to total mononuclear cells, with the number of CD34+ cells in parentheses. BMMC indicates bone marrow mononuclear cells; CHF, congestive heart failure; NYHA, New York Heart Association classification; ESV, end-systolic volume; EF, ejection fraction; MBF, myocardial blood flow; EDV, end-diastolic volume; $\dot{V}O_2$, maximal total body oxygen consumption; and MRI, magnetic resonance imaging.

provide a more definitive test of the efficacy of myoblast therapy in the setting of chronic ischemic heart disease.

Bone Marrow: The Second Wave of Clinical Trials

Fueled in part by hopes for cardiac transdifferentiation, as well as by the considerable body of data supporting angiogenic activity,⁷¹ bone marrow studies moved remarkably quickly from small animals to clinical trials. This contrasts markedly to the “decade of deliberation,” which preceded initiation of the skeletal muscle clinical trials cited above. The rapid progress of bone marrow studies was facilitated by the extensive clinical experience already in place for marrow as a cell-based therapy. In addition, the large numbers of unfractionated cells that could be obtained with little processing also reduced both cost and regulatory burden. In this regard, it is important to remember that bone marrow is a heterogeneous tissue, containing rare hematopoietic⁷² and mesenchymal⁷³ stem cells ($\approx 0.01\%$ of the total cell population) as well as large populations of committed progenitor cells and their highly differentiated progeny. When considering studies involving unfractionated marrow, it is therefore important to keep in mind that this population contains $>99.9\%$ committed cells, the composition of which may vary from study to study.

Whereas skeletal myoblast trials focused exclusively on chronic ischemic disease, $\approx 50\%$ of bone marrow trials have involved intracoronary infusion in patients with acute myocardial infarction. A summary of bone marrow clinical trials is provided in Tables 2 and 3. Taken collectively, studies of intracoronary bone marrow cell injections in patients with acute myocardial infarction have generally reported beneficial effects, manifest as an improvement in perfusion, tissue viability, and/or function. As with the early trials of skeletal muscle, we must be cautious in interpreting these studies. Most have been observational or have used historical (non-randomized) control groups. The concomitant revascularization (PTCA and stenting) of the cell-grafted target area also

represents a major confounding factor in the interpretation of these results. Strauer et al⁷⁴ reported the first delivery of intracoronary mononuclear cells to patients ≈ 7 days after myocardial infarction and compared these results with those of noninfused patients who refused entry into the trial. Although there was no change in ejection fraction, there was an overall improvement in the extent of hypokinesia/dyskinesia in the treated hearts that was absent in the control hearts. They also measured an increase in thallium uptake after cell infusion compared with the precell baseline but were not able to perform this study in control patients. Assmus et al⁷⁵ studied intracoronary infusion of either unfractionated marrow mononuclear cells or progenitor cells derived from peripheral blood (obtained by adherence to fibronectin and 3 days of culture with vascular endothelial growth factor). Cells were infused ≈ 4 days after myocardial infarction, and results were compared with matched historical controls. They reported that ejection fraction in the 2 cell groups improved from 52% to 60% over 4 months of study, whereas in controls, ejection fraction improved from 51% to 54%. They also reported increases in myocardial perfusion, coronary flow reserve, and glucose uptake after cell infusion, although these measurements could not be made in control patients. No differences between 2 therapeutic cell populations were noted.

To date, only 2 randomized, controlled studies regarding bone marrow cells in cardiac repair have been published. The BOOST trial⁷⁶ included 60 patients, of which 30 received intracoronary injections of unfractionated mononuclear cells an average of 6 days after occlusion, whereas 30 patients formed the control group (but did not get sham marrow aspiration or cell infusion). MRI showed that the cell therapy group had a significant increase in ejection fraction compared with controls (50.0% to 56.7% in treated versus 51.3% to 52% in controls), associated with nonsignificant trends for improved end-diastolic and end-systolic volumes.

In the second study, Chen et al⁷⁷ performed intracoronary delivery of autologous bone marrow-derived mesenchymal

TABLE 3. Bone Marrow Trials in Acute Myocardial Infarction

Study	Cell Dose and Type	Controls	Result
Strauer et al ⁷⁴ (n=10)	9×10 ⁶ to 2.8×10 ⁷ BMMCs (1.9×10 ⁵ to 5.9×10 ⁵ CD34+) with overnight culture given ≈7 days after occlusion	Patients who refused entry into cell study (n=10). No sham harvest or infusion.	<ul style="list-style-type: none"> ● EF unchanged ● Enhanced wall motion (reduced region with functional deficit) ● Trend toward enhanced infarct wall velocity ● Enhanced thallium uptake in cell group (no controls)
Assmus et al ⁷⁵ (TOP-CARE) (n=19)	2.4×10 ⁸ BMMCs (7.4×10 ⁶ CD34+)* given ≈4 days after occlusion	Nonrandomized, matched patients. No sham harvest or infusion. (n=11)	<ul style="list-style-type: none"> ● No difference between cell types ● EDV unchanged ● ESV reduced ● Improved EF (8%) ● Increased MBF, CFR, FDG uptake late vs early (no controls for these end points)
Wollert et al ⁷⁶ (BOOST) (n=30)	2.4×10 ⁹ BMMCs (9.5×10 ⁶ CD34+) given ≈6 days after occlusion	Randomized, concurrently enrolled. No sham harvest/infusion. (n=30)	<ul style="list-style-type: none"> ● Cardiac MRI end points ● Trend toward increased EDV and decreased ESV ● Improved EF (6%)
Chen et al ⁷⁷ (n=34)	4.8–6.0×10 ¹⁰ MSCs given ≈18 days after occlusion	Randomized, concurrently enrolled. Controls received sham harvest and saline infusion. (n=35)	<ul style="list-style-type: none"> ● Improved wall motion and velocity ● EDV and ESV reduced ● EF improved (14% above controls) ● Contractility index† improved ● Increased FDG uptake ● Improved electromechanical function by NOGA early vs late (no controls for this)

Cells were delivered by an intracoronary catheter with balloon occluder to increase dwell time.

*BMMCs or progenitors from peripheral blood (dose not described). Progenitors isolated as fibronectin-adherent cells and cultured ×3 days in presence of VEGF and atorvastatin.

†Contractility index defined as (end-systolic pressure)/(end-systolic volume). BMMCs indicates bone marrow mononuclear cells; MSCs, bone marrow mesenchymal stem cells; CFR, coronary flow reserve; FDG, ¹⁸F-fluoro-deoxyglucose positron emission tomography. Other abbreviations as in Table 2.

cells in 34 patients an average of 18 days after the revascularization procedure. Although derived from bone marrow, mesenchymal stem cells differ significantly from hematopoietic stem cells in that they can form bone, fat, and cartilage, with some evidence for forming occasional cardiomyocytes after intracoronary infusion.⁴⁶ Furthermore, there is evidence that mesenchymal stem cells can induce local immune tolerance and hence may be tolerated after allogeneic transplantation.⁷⁸ A large dose of 48 to 60 billion cells was infused under 10 atm pressure. Results were compared with those of 35 control patients, who underwent a bone marrow harvest but received only saline infusion. Mesenchymal stem cell infusion resulted in increased fluorodeoxyglucose uptake, an improvement in wall motion, a reduction in ventricular end-systolic and end-diastolic volumes, and a net increase of 14% in ejection fraction compared with a saline-infused control group.

Most recently, at the 2005 American College of Cardiology meeting, late-breaking results were announced for a double-blind, randomized, placebo-controlled trial from Belgium (S. Janssens et al, unpublished observations). Thirty-two patients received intracoronary unfractionated bone marrow cells within 24 hours of acute infarction, compared with 34 patients who received marrow harvest but placebo infusion. MRI showed that marrow cell infusion was associated with greater infarct shrinkage (wound contraction), suggesting that enhanced infarct repair might be one mechanism of benefit. It should be noted, however, that the 2 groups did not differ in ejection fraction.

Other clinical indications for catheter-based bone marrow cell injections have included refractory ischemia⁷⁹ and heart failure.⁸⁰ In these settings, cells were delivered through an endoventricular catheter guided by electromechanical mapping (NOGA). The authors of these pilot experiments have reported striking improvements in outcomes, but this enthusiasm must be tempered by the small size of the investigated patient populations and the lack of control groups. The same limitation applies to the few studies that have entailed surgical transplantation of bone marrow mononuclear cells⁸¹ or CD133⁺ progenitors⁸² into chronic postinfarction scars.

Several points can be taken from these early human trials of bone marrow for cardiac repair. The first is that direct injection or intracoronary infusion of bone marrow cells appears to be feasible and safe in the setting of either acute myocardial infarction or chronic ischemic heart disease. The observation in dogs that intracoronary delivery of mesenchymal stem cells caused patchy microinfarcts⁸³ has not been reported for humans, although investigators need to remain watchful. Second, most studies offer evidence that bone marrow cell transplantation can improve either myocardial contractile function or perfusion, with several reports of both. No clear consensus emerges when one examines cell dose, however. Anywhere from 9×10⁶ cells to 6×10¹⁰ cells were delivered, representing a remarkable 6700-fold range in cell dose. Dosing studies will need to be performed as the field moves ahead. Finally, it is not clear whether the optimal cell population to infuse is a hematopoietic stem cell, a mesenchymal stem cell, other rare progenitors, or the much more

abundant committed cells. One of the major goals for bone marrow studies will be to identify the therapeutic cell population from these complex mixtures. Taken together, we think that these data support continued clinical studies of bone marrow cells, with appropriate controls, to determine efficacy, explore the mechanism of action, and ensure that there are no unexpected complications (eg, acceleration of atherosclerosis/restenosis).

Translating From Bench to Bedside

It can always be questioned when an emerging area of science is ready to move from preclinical to clinical studies. Basic scientists generally strive to establish a solid understanding of the mechanism through which an intervention works, in addition to the obvious issues of feasibility, safety, and efficacy from animal studies. Because one seldom understands any therapy completely (we are still learning about aspirin), strict adherence to this mindset can delay implementation of an otherwise useful new treatment. Clinical researchers, conversely, may be less concerned with detailed mechanisms and more focused on the unmet clinical needs of their patients. Excessive zeal to test the latest promising strategy can also be problematic, if it brings therapies to the clinic before there is sufficient scientific evidence for safety or efficacy.

With these caveats in mind (and at the risk of offending both groups of investigators), we propose the following as criteria for translating a cell-based trial to the clinic.

1. Reproducible preclinical demonstration of safety and efficacy in multiple laboratories.

Reproducibility is a key element of any therapeutic intervention. If a finding cannot be reproduced by other professionals in a highly controlled laboratory setting, there is little hope for a treatment succeeding in the more variable world of human clinical trials. Conversely, any intervention capable of eliciting a robust effect will inevitably be reproduced by multiple groups.

2. A "reasonable" degree of mechanistic understanding.

Admitting that no clinical intervention can be completely understood, it is still critical that interventions be based on clearly stated hypotheses, validated at the molecular, cellular, and animal model levels before starting clinical studies. Ideally, these hypotheses should permit testing and refinement in human trials, eg, through imaging or pathology-based approaches. Understanding the mechanisms underlying functional improvement in response to cell therapy, at least in part, is required if one hopes to design rational experimental and/or clinical studies to enhance the efficacy of the intervention.

3. Validation in scaled-up, physiologically relevant large-animal model whenever possible.

Although small animals offer the advantages of low cost, genetic malleability, and high throughput, they cannot reproduce many aspects of human cardiovascular physiology. For example, the high heart rate of mice or rats could prevent detection of pacemaker activity in stem cell implants, whereas grafting the same cells into dogs, pigs, or sheep could allow this complication to be detected. Furthermore, large animal models may identify problems or advantages

associated with scale-up that would not be obvious from mouse studies. Acute myocardial infarction can be modeled reasonably well in large animals, which should permit the best possible testing for feasibility, safety, and efficacy.

Experimental and Clinical Cell Transplantation: Lessons Learned

Although there is clearly much to be sorted out in the area of stem cell–based myocardial repair, several lessons are emerging. From a mechanistic standpoint, it is clear that improved physiological function is not tantamount to myocardial regeneration. Preclinical studies indicate that functional improvement after transplantation of fibroblasts, smooth muscle cells, skeletal muscle, and endothelial progenitors is unlikely to result from newly generated, beating cells. Indeed, using contractile function alone, one could mistakenly conclude that ACE inhibition or β -blockade regenerated the heart. Rather, proof of regeneration requires a combination of structural, physiological, and molecular end points. We would suggest that one must demonstrate newly created cardiomyocytes, electromechanically coupled with host myocardium, to prove that heart regeneration has been achieved.

A second lesson is quite encouraging and may have more important implications for patients in the near term: cell-based approaches can improve function of the injured or failing heart independently of true regeneration. Although bone marrow clearly does not generate significant numbers of new cardiomyocytes, there is very good preclinical evidence for a physiological benefit when it is delivered in an acute or chronic myocardial infarction setting. This benefit may relate to enhanced angiogenesis/arteriogenesis, to reductions in ventricular remodeling, or to cytokine-mediated effects that enhance survival of resident cells, just to name a few examples.

A final lesson is the current difficulty of deriving definitive cardiomyocytes from adult progenitors. Skeletal myoblasts, hematopoietic stem cells, and bone marrow mesenchymal stem cells are all examples of cells originally postulated to generate new myocardium but whose beneficial effects are now thought to result from noncardiogenic mechanisms. This should not be taken to mean that there is no hope for adult stem cells, because additional candidate cardiomyocyte progenitors continue to emerge, eg, from the marrow,⁸⁴ the fat,⁸⁵ and the heart itself.^{56–58,60} Although this and other areas of adult stem cell biology definitely need to be pursued further, it seems logical also to pursue the goal of repairing the heart with cardiomyocytes themselves. This notion is strongly supported by the electrophysiological demonstration of the integration of transplanted fetal cardiomyocytes within the recipient myocardium, as evidenced by the synchrony in calcium transients between donor and recipient cells.⁸⁶ Because ethical and practical constraints limit the use of fetal tissue for any clinical application, we submit that a significant effort should be applied toward research on embryonic stem cells for cardiac repair. It has recently been shown that human embryonic stem cell–derived cardiomyocytes can serve as biological pacemakers after transplantation into the left ventricle of swine⁸⁷ and guinea pigs.⁸⁸ This implies stable integration and electromechanical coupling with host myo-

cardium. Furthermore, human embryonic stem cell–derived cardiomyocytes have recently been shown to proliferate extensively after implantation in the rat heart,⁸⁹ raising the possibility for substantial expansion after delivery. It is clear that embryonic stem cell research carries its own challenges in terms of ethics and technique (propagation, cardiomyogenic precommitment, immunogenicity), but if these challenges can be overcome, the scalability of embryonic stem cells⁹⁰ could serve as a virtually unlimited source of cells for true myocardial replacement therapy.

Future Perspectives

For clinical trials for cell-based cardiac repair, it is fair to say “this train has left the station.” It is not a question of whether cell therapies will be tried in patients but rather how they will be tried. If the initial studies of autologous adult myoblasts and bone marrow continue to demonstrate safety, it seems likely that investigators will move to more complex cells with potentially greater benefit. What might these be? An ideal cell population would (1) be available as a standardized “off the shelf” reagent; (2) generate new cardiomyocytes; (3) proliferate after implantation to repopulate large expanses of damaged myocardium; (4) generate a new coronary vasculature, either through direct incorporation or by stimulation of host cells; (5) be tolerated by the immune system; and (6) minimize the amount of scar tissue at the graft–host interface. It is clear that no cells at present meet all these criteria, but key elements are starting to come together. For example, human embryonic stem cells give rise to definitive cardiomyocytes that proliferate after implantation.⁸⁹ Marrow-derived mesenchymal stem cells will home to areas of injury^{91,92} and may be tolerated allogeneically.⁷⁸ Endothelial progenitor cells also home to areas of injury or chronic ischemia and increase local tissue perfusion.^{93–95} A next logical step to attain increased benefit is to combine various cell populations, eg, stem cell–derived cardiomyocytes plus endothelial progenitor cells, to target both the muscle and vascular compartments. Progress is being made in generating “universal donor” cells by knocking out immunogenic loci such as human leukocyte antigen or engineering cells to express local immunosuppressant molecules.⁹⁶ A complementary approach is to induce allotolerance through bone marrow chimerism (by cotransplanting immune-regulating hematopoietic cells specific to the solid tissue type).⁹⁷ If successful, allogeneic transplantation could greatly simplify the cell production phase by permitting large-scale standardization of cell preparations, rather than the case-by-case approach currently required for autologous cells.

As cells with greater potential for differentiation are used, they will bring with them increased risk. Arguably, the 2 most feared complications from cell therapy are arrhythmogenesis and tumor formation. Investigators in cell-based cardiac repair cannot repeat the errors in judgment that plagued the gene therapy field 5 to 10 years ago.⁹⁸ Given that many patients with acute infarcts do quite well with standardized therapies, these may not be the best people in whom to perform initial feasibility and safety studies with novel cell populations. “No-option” patients with end-stage heart failure stand to gain the most from an intervention, and these may be

the patients for whom some cell trials are best suited.⁹⁹ Furthermore, for some analytical end points, the subset of end-stage heart failure patients undergoing placement of ventricular assist devices as a bridge to transplantation can be extremely informative. Cells can be delivered at the time of device placement, and the patients can be studied by imaging techniques while they are waiting for their new hearts, allowing determination of myocardial blood flow, metabolism, and so on. (Of course, assessment of contractile function is limited while the device unloads the heart.) After heart transplantation, the cell-engrafted native heart can be studied histopathologically and by cellular and molecular approaches. Adverse complications will be mitigated by the presence of the assist device, which can maintain cardiac output even if the native heart is fibrillating. In the event of a cardiac tumor, cardiectomy at the time of transplantation should prove a definitive therapy.

Finally, one can achieve some perspective by comparing the emerging field of cardiac repair with the more mature field of bone marrow stem cell transplantation. When bone marrow transplantation was first attempted in humans in the 1950s, it met with frequent episodes of graft failure (reviewed by Diaconescu and Storb¹⁰⁰). As techniques to improve engraftment were developed, many transplant patients then succumbed to graft-versus-host disease. Spurred on by occasional successes, however, investigators returned to the laboratory to develop better models and eventually found that dogs predicted human responses much better than did mice. Armed with a reliable model, successful regimens for bone marrow transplantation were finally developed, nearly 2 decades after the initial attempts. Nowadays, bone marrow and peripheral blood stem cell transplantation are standard regimens that save tens of thousands of lives worldwide each year. With clinical trials of cell-based cardiac repair just getting under way, there is a great deal of excitement, and expectations are high. We need to take a long-term view, however, and if these initial trials are not optimally successful, we must return to the laboratory to refine our hypotheses. It may require close interaction between clinical investigators and basic scientists over a decade before we have optimized techniques for rebuilding the heart. But if we succeed, cell-based cardiac repair will offer hope for millions of patients worldwide each year who would otherwise suffer from inexorable progression of heart failure.

Note Added in Proof

Since the preparation of this manuscript, Dib et al¹⁰¹ have reported a 4-year follow-up of 30 patients treated with autologous myoblast cell transplantation at the time of bypass grafting or ventricular assist device implantation. Overall, this study provides additional evidence for the feasibility and safety of the procedure, but it shares with the other phase I trials the limitation inherent in the lack of a control group, and thus the inability to draw meaningful conclusions about efficacy. Of particular interest, however, are the pathological findings made in 4 of 6 of these patients who received myoblasts at the time of left ventricular assist device implantation and whose hearts could then be retrieved during subsequent transplantation. The demonstration of mature

skeletal muscle in these patients' hearts confirms previous observations⁶⁹ of myoblast engraftment in human scarred myocardium.

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