Heart failure is a highly prevalent, debilitating, and costly condition with generally poor clinical outcomes. Aside from heart transplantation, which is an available treatment option for only a small fraction of patients because of donor organ shortage, there is no effective therapy that can reverse the course of this disease. A single episode of myocardial infarction may result in the loss of 1 billion cardiomyocytes or more (≈25% of total cardiomyocytes). Given the limited intrinsic capacity of the adult heart to repair itself, the goal of cardiac regenerative medicine has centered on strategies to remuscularize the diseased heart.

Conceptually, the functional regeneration of an infarcted heart would entail the replacement of lost myocardium by aligned, electrically coupled, and mature new cardiomyocytes that beat in synchrony with the host myocardium. Beyond achieving this remarkable result, the avoidance of procedure-related complications and other potential adverse events, such as tumor formation or cardiac arrhythmia, is paramount for the therapy to be considered a success. While the process of finding the most appropriate cell type and delivery approach to achieve this objective has been the “holy grail” of cardiac regenerative medicine, a growing body of literature has now documented our initial efforts in this area. From these studies, the encouraging finding is that cell transplantation into the diseased heart (via intracoronary, transendocardial, or direct epicardial injection) appears to be reasonably safe. Furthermore, the practicalities of harvesting, expanding, and reintroducing cells back into the patient do not seem too cumbersome. However, the sobering reality we have learned is that tremendous roadblocks exist in achieving significant improvement in long-term cardiac function and bona fide remuscularization after cell transplantation.

We believe the field of cardiac regeneration is at a crossroad. Although ongoing debate regarding the most appropriate cell type, timing, route of delivery, and clinical setting will be addressed by further experimentation in animal models and patients, we need to consider whether the premise of cell transplantation as a treatment strategy for diseased hearts is still fundamentally sound and viable for further exploration. As we now enter the second decade of research on cell-based therapy for cardiovascular disease, it is instructive to revisit some of the key findings from published cell transplantation studies to better understand what is needed to move the field forward. We briefly summarize the efforts related to the transplantation of autologous noncardiac cell populations, such as skeletal myoblasts and bone marrow–derived cells, as well as recent trials on resident cardiac stem/progenitor cells (Figure). We also discuss whether pluripotent stem cells such as human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) will be able to move into the clinical arena in the next decade, and the advantages and disadvantages of these cells in comparison with autologous adult-derived cells. Ultimately, our pursuit of cardiac regeneration will be looked on by future generations as akin to Ponce de León’s search for the Fountain of Youth, or as one of the greatest success stories in modern medicine. It is our hope that the combined efforts of many dedicated cardiovascular investigators in this area will eventually lead to a durable therapy that can reverse the increasing incidence of ischemic heart failure.

Skeletal Myoblasts

The initial observation that skeletal myoblasts can be harvested and cultured ex vivo from muscle biopsy samples and then transplanted into an infarcted animal heart sparked the interest of basic and translational investigators that cell-based therapy may be a potentially viable strategy for cardiac remuscularization. The appeal for using skeletal myoblasts as a donor cell source is their autologous origin, ability to rapidly expand in culture, and propensity to generate muscle cells by spontaneous differentiation. Furthermore, these cells appear to engraft into the injured heart with remarkable efficiency and undergo in situ differentiation into striated muscle bundles. Although the earliest clinical studies conducted in small numbers of patients using autologous skeletal myoblasts reported significant improvements in cardiac function, a subsequent trial with a larger number of participants found no demonstrable benefit (as measured by ejection fraction) and a high prevalence of ventricular tachyarrhythmia requiring the implantation of defibrillators. This early effort with skeletal myoblast transplantation illustrates our capability to move quickly from basic discovery to human studies in the cell therapy arena. However, the finding of potentially lethal arrhythmia in the remuscularized hearts suggests the requirement of cell-to-cell coupling between the transplanted graft and endogenous cardiomyocytes to minimize electric heterogeneity within the diseased heart. The introduction

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of connexin 43, a cell junction protein involved in cardiomyocyte coupling, into mouse skeletal myoblasts reduced arrhythmia after cell transplantation.7

**Bone Marrow–Derived Stem Cells**

The finding that hematopoietic cells may harbor greater developmental plasticity than previously suspected had spurred the subsequent investigation of these cells for regenerative studies in other tissues, including the heart.8 Studies on hematopoietic cells have most often used unselected mononuclear cells isolated directly from the bone marrow or from peripheral blood, as well as a more refined subset of marrow–derived or circulating cells termed endothelial progenitor cells. This latter subset has been reported to induce neovascularization in animal models9 and are enriched in cell populations possessing the cell surface markers CD34, CD133, or receptors for vascular endothelial growth factor. Conceptually, the introduction of autologous cells that exhibit stem cell features into a damaged heart is highly appealing. Furthermore, results from clinical trials have supported the safety and feasibility of intracoronary delivery of bone marrow and circulating stem cells. However, despite encouraging results in animal models, the efficacy of bone marrow cell transplantation in patients has been modest overall and inconsistent between studies.10–17 Nevertheless, these initial studies have illustrated 3 key issues that warrant further consideration: (1) The low retention rate of bone marrow cells in the heart; (2) The questionable efficiency of cardiomyocyte transdifferentiation; and (3) The uncertain mechanisms of functional improvement by the delivery of bone marrow cells.

It appears that the chosen cell type, as well as the route of administration, may play a role in the issue of low cell retention in the heart after transplantation. One human positron emission tomography imaging study demonstrated that CD34+ cells homed to the infarcted myocardium with ≈10-fold greater efficiency compared with unselected bone marrow–derived cells after intracoronary cell transfer.18 Additionally, emerging data suggest that direct intramuscular injection may be slightly more effective than intracoronary delivery.19,20 For intracoronary injections, the requirement for diapedesis through the coronary arterial wall may account for the limited amount of cell retention. Hence, innovative strategies aimed at increasing the targeting efficiency of transplanted cells and methods promoting cell survival after transplantation into the heart would prove highly beneficial.21

The efficiency of cardiomyocyte transdifferentiation from bone marrow–derived cells still poses significant challenges. Although the initial findings appeared highly encouraging, subsequent studies identified several confounding issues, such as cell fusion and imaging artifacts that may account for some, if not all, of the apparent hematopoietic cell transdifferentiation into cardiomyocytes.22–25 Although it is not improbable that bone marrow cells can transdifferentiate into cardiomyocytes with the introduction of appropriate epigenetic modifiers, the exact conditions and molecular factors required to achieve this in vivo are far from clear.

Despite the low efficiency of cell retention and cardiomyocyte transdifferentiation, there appears to be a modest, but statistically significant (3% to 5%), increase in ejection fraction after bone marrow cell transplantation compared with control.14,26 Head-to-head comparisons and meta-analyses suggest this effect has not been reserved to a particular cell type, although most studies used unselected bone marrow mononuclear cells rather than mobilized circulating cells or other selected cell populations.27–29 There is a growing consensus that the beneficial effects are mediated by paracrine action from either the process of cell injection alone, regardless of cell type, or specific factors secreted by the transplanted cells, or both.30,31 The presence of these factors may exert a favorable remodeling effect, augment neovascularization, or stimulate the expansion of endogenous cardiac progenitor cells. In support of this, Loffredo et al31 showed recently that bone marrow–derived c-kit-positive cells stimulate cardiomyogenesis by increasing the number of stem/progenitor cell–derived cardiomyocytes. Furthermore, the number of proliferative BrdU+ cardiomyocytes increased in bone marrow c-kit-positive but not c-kit-negative cell–treated hearts. These results suggest that the identification of specific paracrine factor(s) and the targeted cell population mediating endogenous cardiomyogenesis may allow us to achieve at least as comparable a regenerative response as bone marrow cell transplantation, but in a cell-free manner.
Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of mesodermally derived tissues and constitute another potential candidate for cell-based therapy. For cardiac applications, they have been most commonly isolated from the bone marrow or adipose tissue and defined by their ability to adhere to plastic culture dishes during in vitro propagation. In vitro, MSCs may be induced to exhibit cardiomyocyte-like features in the presence of the demethylating agent 5-azacytidine or when cocultured with cardiomyocytes.\(^3\)\(^2\)\(^-\)\(^4\) They also have the potential advantages of possessing low immunogenicity, as well as the ability to home to the site of injury within the myocardium.\(^5\)\(^6\) The combination of these attributes, in theory, could allow for intravascular allogeneic delivery of cell therapy via an off-the-shelf approach without the requirement for administering concomitant immunosuppression.

In this regard, the results from the recently reported Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON) study showed that transcatheter allogeneic MSC transplantation was associated with a favorable safety profile when compared with autologous MSCs transplantation.\(^3\)\(^6\) Also, in a previous randomized trial, the introduction of allogeneic MSCs after acute myocardial infarction resulted in an improvement in global symptom score at 6 months and a small but significant improvement in ejection fraction in patients with large myocardial infarctions.\(^3\)\(^7\)\(^-\)\(^9\) As with bone marrow–derived stem cell transplantation, the low frequency of MSC engraftment and cardiomyogenic differentiation in the heart suggest that the functional improvement observed in preclinical models and human trials is likely related to paracrine effects of the injected cells as opposed to generation of de novo cardiomyocytes.\(^3\)\(^8\) Several other ongoing clinical trials, including Prospective Randomized Study of MSC Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS), Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HF), Adipose-Derived Regenerative Cells Delivered Via the Intracoronary Route in the Treatment of Patients With ST-elevation Acute Myocardial Infarction (ADVANCE), and Adipose-Derived Stem and Regenerative Cells in the Treatment of Patients With Non Revascularizable Ischemic Myocardium (PRECISE), will add important information regarding safety and efficacy of bone marrow–derived or adipose-derived MSC therapy in cardiac disease.

Endogenous Cardiac Stem Cells

Although the lesion from bone marrow cell transplantation may be found in the presence of a paracrine-mediated effect in cardiac repair, the goal of achieving cardiomyogenesis by direct cell introduction into the injured heart remains elusive. As the search for a true cardiomyogenic cell population continues, several laboratories have reported an endogenous population of cardiac stem cells (CSCs) or progenitor cells residing in the postnatal heart. Adult CSCs have been isolated based on the expression of surface markers or functional features such as c-Kit, stem cell antigen-1, multidrug resistance gene-1/ATP-binding cassette subfamily G member 2 (also known as side population), and aggregational properties (ie, cardiosphere).\(^3\)\(^0\)\(^-\)\(^2\)\(^2\) The capacity of these CSCs to self-renew in a clonal fashion in vitro and to differentiate into multiple cardiovascular lineages both in vitro and in vivo suggests the potential therapeutic benefit of these cells after transplantation into the injured heart.\(^3\)\(^3\) The first study (Cardiac Stem Cells in Patients With Ischemic Cardiomyopathy [SCIPIO]) evaluating the safety and feasibility of c-Kit-positive CSCs in a clinical context was recently reported.\(^3\)\(^4\) The encouraging finding is that harvesting and ex vivo expansion of c-Kit-positive CSCs appears to be feasible, and no overt toxicity has been found thus far. Although not prespecified as the primary end points, left ventricular systolic function increased and infarct size reduced after intracoronary infusion of autologous ex vivo expanded c-Kit-positive cardiac cells in ischemic heart failure patients. Further studies will be needed to determine whether the functional benefit achieved is attributable to new cardiomyogenesis or paracrine effects as observed in bone marrow cell transplantation. In addition, as the identity and the cardiomyogenic potential of cardiac c-Kit-positive cells becomes clarified between different groups,\(^3\)\(^5\)\(^-\)\(^7\) it will be important to have additional confirmation of the results in SCIPIO by other investigators to help sustain the interest of cardiovascular clinicians and scientists in this approach.

In this regard, a prospective, randomized, phase 1 study of cariosphere-derived cells (CDCs; Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction [CADUCEUS]) was recently reported.\(^3\)\(^7\) Unlike the SCIPIO study in which cardiac cells were further purified based on their expression of c-Kit, CDCs are collected from aggregating cells after right heart biopsy and ex vivo expansion. They express c-Kit in ≈20% of the cells.\(^3\)\(^8\) When introduced into patients with systolic heart failure, CDC-treated patients showed an apparent reduction in scar size and a corresponding increase in heart muscle mass. Regional contractility was increased as well. Interestingly, the end-diastolic and end-systolic volumes and the overall left ventricular ejection fraction (LVEF) were not changed compared with control patients. It remains to be seen whether the transplantation of CDCs results in new cardiomyocyte generation, given the reported cardiomyogenic potential of these cells, or whether an endogenous cardioprotective mechanism becomes activated to prevent myocyte loss. Although LVEF is an imperfect measurement of systolic function, the lack of a significant increase in this parameter, despite the reported reduction in scar size and increase in heart muscle mass, suggests that even if new cardiomyocytes were generated from the transplanted CDCs, their ability to contribute to positive force generation during systole is somewhat limited. Further studies that address the extent of CDC engraftment in the transplanted heart, the degree of their cardiomyocyte differentiation, and the extent of electric coupling with native cardiomyocytes will help clarify the apparent disconnect between the increase in muscle mass and the lack of improvement in LVEF.

Pluripotent Stem Cells

Thus far, our efforts to regenerate the diseased heart have focused on identifying a population of cells that is both autologous and most likely to harbor cardiomyogenic potential.
The immunocompatibility and the relative accessibility of autologous cells have been chosen over cardiomyogenic efficiency. This bias has been deliberate because it allows us to move from preclinical studies to human trials in a relatively short period of time. As we speculate on where cell therapy for cardiac disease might be in another decade, it is worth revisiting our original goal of in vivo cardiomyocyte replacement. There is a general agreement that pluripotent stem cells such as ESCs and iPSCs have unambiguous ability to differentiate into most, if not all, major cell types within the body. Since their initial discovery in 1998,49 human ESCs have been touted as one of the most promising cell sources for regenerative therapies. The ability of human ESCs to self-renew indefinitely in vitro and differentiate into cell types of interest with the supplementation of selected growth factors has stimulated the development of various differentiation strategies to increase the efficiency of cardiomyocyte generation and purification.50

One of the main reasons for the need to generate a high degree of cardiomyocyte purity is the alarming frequency of teratoma formation when undifferentiated ESCs are introduced into the heart.51,52 The issue of tumorigenicity has raised the threshold for United States Food and Drug Administration approval of human ESC products so high that 1 well-recognized company in this area had to eliminate its entire program. There are currently active trials on Stargardt disease and adult macular degeneration using human ESC-derived retinal epithelial cells. It remains to be seen whether teratoma formation will be an issue in these early applications of human ESC-derived products.

For cardiac diseases, a number of human ESC-derived cardiomyocyte (hESC-CM) preclinical studies have been published.21,53 In these studies, the transplantation of hESC-CMs into the injured rodent heart has resulted in small grafts that are largely electrically isolated. As a consequence, the functional benefit observed early (4–6 weeks) after transplantation was not sustained at 12 weeks or beyond. An encouraging finding from these studies is that no teratoma formation was observed despite the presence of ≈20% noncardiomyocytes within the cardiomyocyte-enriched cell population that underwent transplantation. However, because these studies were performed as xenografts in immunocompromised rodent hosts and the number of the engrafted cells has been very small, it remains to be seen whether teratomas would be found when a larger number of these cells are transplanted into humans.

Assuming the teratoma concern can be eliminated in the near future, a patient who undergoes hESC-CM treatment would still require immune suppression given the allogeneic source of the engrafted hESC-CMs. Despite intensive efforts to generate patient-specific hESCs by somatic cell nuclear transfer, there has, so far, been no bona fide hESC line created by this approach. The interest in pursuing the generation of patient-specific human ESCs from somatic cell nuclear transfer waned when it was discovered by Takahashi and Yamanaka that the forced expression of Oct4, Sox2, Klf4, and c-Myc can induce a pluripotent cell phenotype from somatic cells.54,55 These so-called iPSCs exhibit properties highly similar, but not identical, to hESCs and can readily generate cardiomyocytes by in vitro differentiation using protocols similar to those used for hESCs. Although the generation of iPSCs from patients’ own fibroblasts should, in principal, obviate the need for immunosuppression when cardiomyocytes derived from these cells are transplanted, a recent study reported the triggering of an immune response against autologous iPSC-derived teratoma in mice, raising the possibility of rare antigen expression in iPSCs that is not normally expressed in ESCs.56,57 It would be important to clarify whether these antigens are unique to teratoma per se or are present in all iPSC-derived cells. If these antigens are expressed ubiquitously in iPSCs and their progenies, the advantage of autologous human iPSCs over allogeneic hESCs would be limited.

An additional consideration that will undoubtedly play a role in pluripotent stem cell–based therapy for cardiac disease is the phenotype of the iPSC/ESC–derived cardiomyocytes. Myocytes in the heart are naturally diverse, with highly specialized physiological attributes and regional diversity that are essential for normal cardiac function. Currently, all protocols for human iPSC/ESC differentiation generate a mixture of atrial and ventricular cardiomyocytes and nodal-like cells. These cells are largely immature and do not resemble the mature rod-shaped, and often bunucleated, cardiomyocytes found in an adult mammalian heart. Their fetal ion channel expression and electrophysiological properties may potentially be arrhythmogenic if electrically coupled with endogenous mature cardiomyocytes. If so, then toxicity issues will significantly diminish their translational potential in clinical applications.58,59 In this respect, efforts addressing factors that regulate ventricular versus atrial vs nodal-specific differentiation will be highly valuable.60 Furthermore, understanding the key roadblocks that prevent electromechanical maturation of in vitro differentiated iPSC/ESC–derived cardiomyocytes will help to minimize cardiotoxicity from cardiomyocyte transplantation. Ultimately, it will be important to determine the exact degree of cardiomyocyte maturation needed to enable the most optimal engraftment, expansion, and maturation after transplantation. It might be the case that transplanting cardiomyocyte progenitor cells can lead to greater cell engraftment and survival in the diseased heart, but these cells mature poorly and form a heterogeneous population of cardiovascular cells that becomes arrhythmogenic. However, transplanting fully mature cardiomyocytes may result in poor overall engraftment and survival because of their greater demand for oxygen and cell–cell contact despite being more phenotypically compatible with endogenous adult cardiomyocytes. Additional studies in large animal disease models using human ESC/iPSC–derived cardiac progenitor cells, immature cardiomyocytes, and mature ventricular cardiomyocytes should help to resolve some of these dilemmas.

**Direct Cardiomyocyte Reprogramming**

The remarkable success of somatic cell reprogramming into iPSCs has generated a renewed interest in direct cell lineage reprogramming since the discovery of MyoD.61 Indeed, reports of fibroblast conversion into neurons, blood, and liver cells by transcription factor overexpression have been published recently.62–67 The advantage of somatic cell transdifferentiation into another adult cell without an intermediate state
of pluripotency is that it may circumvent the risk of teratoma formation associated with pluripotent stem cell–derived cell transplantation. In this regard, Ieda et al\textsuperscript{68} reported the reprogramming of murine postnatal cardiac and tail tip fibroblasts into cardiomyocyte-like cells by overexpressing a combination of three cardiac transcription factors—Gata4, Mef2c, and Tbx5. Using fibroblasts from α-nyosin heavy chain–green fluorescent protein transgenic mice, ≈6% of virally infected cells were double-positive for green fluorescent protein and cardiac Troponin-T. In rare instances, spontaneous calcium transients were noted in infected, but not control, cardiac fibroblasts. Although in vitro reprogrammed fibroblasts may constitute a novel source of transplantable cardiomyocytes without the risk of teratoma formation, we believe the reprogramming efficiency must improve significantly (eg, up to >50% conversion into cardiomyocytes from a starting pool of fibroblasts) for this strategy to be therapeutically relevant, given the issues of cell retention and survival after transplantation discussed and the lack of ability of these cells to proliferate after transplantation. Nevertheless, the prospect for cellular reprogramming to play a role in cardiac regenerative therapy is quite intriguing, and the development of a robust and reproducible protocol for direct conversion of fibroblasts into cardiomyocytes will be important to move the field forward. If this can be achieved, then we envision the possibility that 1 day we might directly reprogram scar fibroblasts in the injured heart without the need for cell transplantation. The recent reports that in vivo reprogramming of scar fibroblasts into cardiomyocytes appears to be more efficient than in vitro reprogramming is a promising first step toward making this a clinical reality.\textsuperscript{69,70}

Future Perspective

In the past decade, we have witnessed tremendous excitement among basic and translational scientists toward therapeutic strategies that involve direct cell transplantation into the injured heart. From the wealth of preclinical and clinical data gathered, we gained a greater appreciation for the inherent challenges in survival, retention, cardiomyogenic differentiation, and functional integration of cell transplantation. Although no durable therapy has arisen from these efforts thus far, the knowledge we gained with regard to cell procurement, processing, and delivery will be useful for ongoing and future cell transplantation studies and should improve our chances of success with this approach.

Important issues that will continue to require major investigative efforts include the identification of the most effective strategy for cell engraftment and survival, the most efficient delivery technology to accomplish this goal, the most relevant cell type for transplantation that can achieve cardiomyogenesis to the level that directly contributes to positive force generation, and the improvement in hard clinical endpoints such as reduction in mortality and recurrent hospitalization. With regard to translational clinical studies, the challenges of objectively quantifying improvement in cardiac contractility and function in humans will require appropriate trial design and incorporation of technologies that are least susceptible to observer bias. In this regard, it is noteworthy that many of the bone marrow trials reporting improvement in LVEF used echocardiography as the method of functional assessment, whereas studies using cardiac magnetic resonance imaging showed less or no improvement after bone marrow cell transplantation. This suggests that meticulous execution of a double-blinded trial design and the incorporation of cardiac magnetic resonance imaging in the assessment of LVEF will be optimal in future trials. Given the relatively small change in LVEF after cell transplantation, the absence of data on mortality or major adverse cardiovascular events, and the small number of patients studied in each trial thus far, future clinical studies will likely shift away from patients with acute myocardial infarction and toward patients with ischemic heart failure or vascular insufficiency, in whom the benefit from cell therapy may be greater. As we continue the noble pursuit of cardiac regeneration, it will be important to maintain objectivity in the reporting of preclinical and clinical outcomes to prevent the creation of unrealistic expectations from the public, particularly given the low societal tolerance for medical errors.

We believe the future prospect for cardiovascular cell therapy remains bright. The finding of an effective therapy that can remuscularize a damaged heart not only will be a remarkable achievement in modern medicine but also will be the most effective, if not the only, way that we can reverse the growing incidence of heart failure worldwide.

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Disclosures

None.

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