Cell Stem Cell
Perspective

Cardiac Stem Cell Therapy and the Promise of Heart Regeneration

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Stem cell therapy for cardiac disease is an exciting but highly controversial research area. Strategies such as cell transplantation and reprogramming have demonstrated both intriguing and sobering results. Yet as clinical trials proceed, our incomplete understanding of stem cell behavior is made evident by numerous unresolved matters, such as the mechanisms of cardiomyocyte turnover or the optimal therapeutic strategies to achieve clinical efficacy. In this Perspective, we consider how cardiac stem cell biology has led us into clinical trials, and we suggest that achieving true cardiac regeneration in patients may ultimately require resolution of critical controversies in experimental cardiac regeneration.

Introduction

The race is on: throughout the world, basic and clinical investigators want to be the first to identify new approaches to regenerate cardiac tissue and to prove the effects of these therapies in patients with heart disease. Despite substantial progress in treating many types of heart disease, the worldwide heart failure burden will remain enormous through this century. The potential of stem cells and the scope of the heart failure problem have fueled a stampede to be the first to achieve human heart regeneration. Cell transplantation approaches are attractive given their relative ease of use and good safety profile to date, but reproducible results endorsing a specific strategy for routine patient care are lacking. Meanwhile, cellular reprogramming strategies are appealing because they potentially allow precise control over cellular behavior, but much work remains before the safety of reprogramming allows clinical testing. Current clinical trials focus largely on injection of cells with cardiomyogenic potential into the heart; however, given the limitations of this approach, we wonder: is this the path to take right now?

As we consider the current state of the heart regeneration field, it is worth pausing to reflect on the 1960s, when heart transplantation emerged. Initial excitement over heart transplantation led to over 100 heart transplantations worldwide in 1967 and 1968. However, disappointing results soon followed, with only a quarter of the patients surviving more than a few months (Kantrowitz, 1998). Renowned cardiologist Helen Taussig expressed concern in 1969 that it was not yet time for human trials, warning, "…our hope should be that physicians and surgeons will proceed with extreme caution until such time as a cardiac transplant will not announce the imminence of death but offer the patient the probability of a return to a useful life for a number of years" (Taussig, 1969). During the 1970s, few human heart transplants occurred as the number of surgeons willing to perform heart transplants dwindled due to high mortality in the first year after transplants (Kantrowitz, 1998). Only after rigorous research in organ rejection and immunosuppression in the 1980s did heart transplantation become the accepted medical practice that it is today (Kantrowitz, 1998). Unfortunately, limitations in organ supply and other issues allow transplantation in only a minority of patients with heart failure, and transplantation will not be a solution for the growing problem of heart disease.

Half a century after the first human heart transplant, we are now confronted with the new challenge of regenerating damaged hearts in the growing number of patients with heart failure. Will we be following a similar path to that of cardiac transplantation? Despite the enormous potential, it is not clear whether we know enough fundamentals to move forward clinically or how fast we should go. Some investigators contend that we know all we need to know to move forward, while others are less confident. In this Perspective, we consider both established principles and ongoing controversies that guide cardiac regeneration research.

Established Principles

We believe that three fundamental principles of cardiac regenerative biology have now been established. First, multipotent cardiac progenitor cells (CPCs) exist in the embryonic mammalian heart (Moretti et al., 2006; Wu et al., 2006); second, there is creation of a limited number of new heart cells after birth in mammals (Beltrami et al., 2003; Bergmann et al., 2009; Malliaras et al., 2013; Mollova et al., 2013; Senyo et al., 2013); and third, some vertebrates, such as newts (Oberpriller and Oberpriller, 1974), zebrafish (Jopling et al., 2010; Poss et al., 2002), and neonatal mice (Porrello et al., 2011), can regenerate myocardium following experimental injury. In an often-controversial field, the establishment of these three principles from different lines of evidence by different laboratories represents seminal progress.

Multipotent CPCs Exist in the Mammalian Embryo

During embryonic development, CPCs arise from a subpopulation of mesodermal precursors that can be modeled from in vitro differentiated embryonic stem cells (ESCs) (Kouskoff et al., 2005). The expression of FLK1 marks a panmesodermal cell population that can give rise to cells in both the primary and secondary heart fields (Kattman et al., 2006) as well as skeletal muscles in the head, neck, and trunk (Motoike et al., 2003). For the primary heart field, a population of bipotential

Table 1. Estimated Rates of Cardiomyocyte Renewal in Adult Mammals

Annual Rate of Cardiomyocyte Renewal	Species	Method	Reference
0.5%–1.9%	human	^{14}C, accelerator mass spectrometry	Bergmann et al., 2009
10%–40%	human	Ki67, phospho-H3, Aurora B, and IdU	Kajstura et al., 2010
7%–23%	human	^{14}C, accelerator mass spectrometry	Kajstura et al., 2012
0.04%–4.5%	human	Phopho-H3	Mollova et al., 2013
1.3%–4%	mouse	BrdU	Malliaras et al., 2013
0.74%	mouse	^{15}N, imaging mass spectrometry	Senyo et al., 2013
1.09%	mouse	[^{3}H]thymidine	Soonpaa and Field, 1997; Soonpaa et al., 2013

KIT+ (also referred to as c-kit+)/NKX2.5+ progenitor cells gives rise to myocardial and smooth muscle cells (Wu et al., 2006). For the secondary heart field, ISL1+ progenitor cells have been described to undergo multilineage differentiation into myocardial, smooth muscle, and endothelial cells (Moretti et al., 2006). Taken together, these studies provide unequivocal evidence for the existence of multipotent progenitor cells in the developing embryo heart. Understanding the mechanisms of embryonic development—in particular, identifying the signals that initiate and terminate heart development—will be crucial to establishing therapeutic regenerative approaches that utilize similar molecular pathways.

Postnatal Cardiomyocyte Renewal Occurs in Mammals, Including Humans

The classic 20th century teaching was that mammalian cardiomyocytes cease replication soon after birth, with subsequent growth of the heart attributed to cardiomyocyte hypertrophy rather than hyperplasia. In the 1990s, the Anversa laboratory provided crucial evidence that mammalian cardiomyocytes not only enter the cell cycle in adulthood, but can also subsequently undergo karyokinesis and cytokinesis (Kajstura et al., 1998; Quaini et al., 1994). Recent studies definitively demonstrate that cardiomyocyte turnover occurs throughout life in mammals, including humans, although estimates of the rate of cardiomyocyte turnover vary dramatically.

Perhaps the most stunning evidence for cardiomyocyte regeneration in humans was revealed by retrospective isotope dating studies. Taking advantage of the dramatic spike and decline of worldwide atmospheric carbon-14 (^{14}C) levels during the 1950s to 1960s due to above ground nuclear bomb testing, Frisen and colleagues developed an ingenious approach to determine the birth date of cardiomyocytes in humans by measuring nuclear ^{14}C content (Bergmann et al., 2009). Their data showed that new cardiomyocytes form in human myocardium at a rate of approximately 1.5% per year at age 25 years, decreasing substantially in the latter half of life (Bergmann et al., 2009).

Using the ^{14}C method developed by the Frisen group, Anversa and colleagues arrived at much higher values for cardiomyocyte turnover in humans (7%–23% per year); in addition, they reported the surprising finding that cardiogenesis increases with age (Kajstura et al., 2012). Mathematical modeling assumptions in the ^{14}C method could explain some of the differences in the ^{14}C studies.

Multiple additional lines of evidence support a low rate of mammalian cardiogenesis and that the rate declines further with age (Table 1). Earlier studies using [^{3}H]thymidine in adult mice estimated an annual renewal rate of approximately 1% per year (Soonpaa and Field, 1997), almost identical to the rates of cardiogenesis estimated by more recent mouse studies (Malliaras et al., 2013; Senyo et al., 2013). A similar rate of cardiogenesis in young human adults was recently confirmed (1.9% at 20 years) using an imaged-based assay in tissue samples procured from donor hearts prior to transplantation (Mollova et al., 2013). Thus, while all studies reveal cardiomyocyte renewal in postnatal mammals, the majority of studies indicate that this rate is very low, on the order of 1% per year, and that the rate declines with age.

Myocardial Regeneration Occurs after Injury in Certain Vertebrates

Critical insight into how we might regenerate human hearts has arisen from vertebrates that can indisputably regenerate myocardium following injury. Urodele amphibians such as newts can survive after amputation of the apical myocardium and demonstrate cardiomyocyte regeneration by 30 days postamputation (Oberpriller and Oberpriller, 1974). Similarly, in zebrafish, amputation of the apex of the heart leads to complete regeneration (Poss et al., 2002). This dramatic regeneration in urodele amphibians and zebrafish is thought to be due to limited dedifferentiation of mature cardiomyocytes and reentry into the cell cycle (Laube et al., 2006). This is supported by evidence of sarcomere disassembly (Jopling et al., 2010) as well as expression of Gata4, a transcription factor that is normally expressed during embryonic development to regulate myocardial formation (Kikuchi et al., 2010).

Studies investigating mammalian cardiomyocyte mitosis after injury can be found as early as the 1970s (Rumyantsev, 1974), although more definitive evidence for the potential of embryonic and neonatal mammalian myocardium to regenerate has recently emerged. Using an elegant mouse model to effectively damage 50% of the developing cardiac tissue by inactivating the gene encoding holocytochrome c synthase, Cox and colleagues demonstrated that lost myocardium is replaced by healthy tissue during fetal development, resulting in only 10% of the cardiac volume occupied by diseased tissue at birth (Drenckhahn et al., 2008). Furthermore, Sadek and colleagues showed that the 1-day-old neonatal mouse heart is capable of regeneration after resection of approximately 15% of the ventricle at the apex (Porrello et al., 2011). This neonatal mouse heart regeneration appears to occur as a result of dedifferentiation followed by proliferation of preexisting cardiomyocytes. However, the ability to regenerate myocardium is rapidly lost by 7 days after birth; instead, the heart develops fibrotic scars similar to the response observed following myocardial injury in

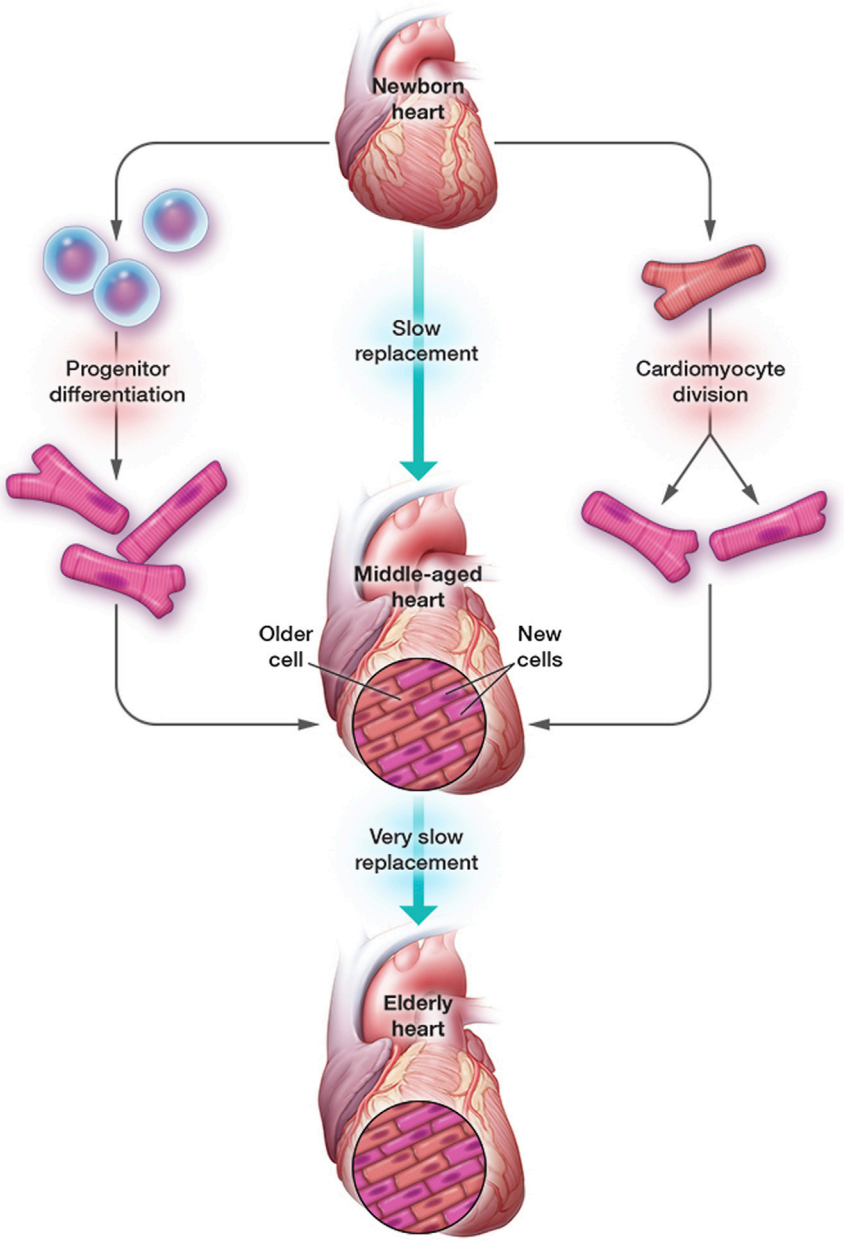

Figure 1. Mammalian Cardiogenesis during Aging

Multiple lines of evidence exist for the refreshment of cardiomyocytes during aging in mammals, with two predominant mechanisms proposed to explain the source of new cardiomyocytes during aging: (1) progenitor cells that give rise to new cardiomyocytes exist in the heart throughout life or (2) mature cardiomyocytes undergo partial dedifferentiation, reenter the cell cycle, and proliferate into new cardiomyocytes. Results from the majority of investigators suggest that this turnover rate occurs at a low level (approximately 1% per year in young adults) and declines even further with age.

adult mice and humans (Porrello et al., 2011). These experiments raise the critical question of what prevents mouse heart regeneration after the first days of life, and point to this first week of life as a crucial period for understanding inherent regenerative mechanisms in mammals.

Unresolved Questions

Though not all encompassing, here we discuss five substantial controversies that will require resolution as we push forward to achieve true cardiac regeneration in a clinical setting. First, we must understand the source of regenerated cardiomyocytes during aging and injury. Second, we must establish the ideal cell source for cell transplantation. Third, we must describe the mechanism by which cell transplantation clinical trials have demonstrated some efficacy. Fourth, we must identify the best therapeutic approach for clinical cardiac regeneration. Finally, we must determine the ideal method to promote stable differentiation of nonmyocytes into cardiac myocytes.

What Is the Source of Regenerated Cardiomyocytes?

Two theories emerged over the past decade to explain the origin of new cardiomyocytes in adult mammals: (1) a progenitor or stem cell gives rise to new cardiomyocytes, or (2) mature cardiomyocytes reenter the mitotic cell cycle to give rise to new cardiomyocytes (Figure 1). There are data to support both of these hypotheses: putative adult progenitor cells in the myocardium have been identified by multiple markers, including c-kit (Beltrami et al., 2003; Fransioli et al., 2008), SCA1 (Oh et al., 2003), and the so-called "side population" cells (Pfister et al., 2005) (more extensively reviewed by Bollini et al., 2011). However, other data suggest that the dominant mechanism of cardiomyocyte generation is not from progenitor cells, but instead from preexisting cardiomyocytes (Senyo et al., 2013). Although these hypotheses are not mutually exclusive, it is likely that one mechanism will ultimately prove dominant in the uninjured mammalian heart.

It is possible that theories of cardiomyocyte refreshment will parallel those of other fields influenced by the explosion of stem cell science, where early reports of adult stem cells as the source of renewal were not supported by later lineage mapping experiments. For example, pancreatic beta cells were thought to arise from progenitor cells, but rigorous lineage mapping studies revealed that beta cells themselves are the dominant source of new beta cells (Dor et al., 2004). Lineage mapping experiments using several markers for putative cardiac progenitors are now underway in many laboratories, and it is likely that these experiments in aggregate will reveal or exclude an important role for adult CPCs in mammals.

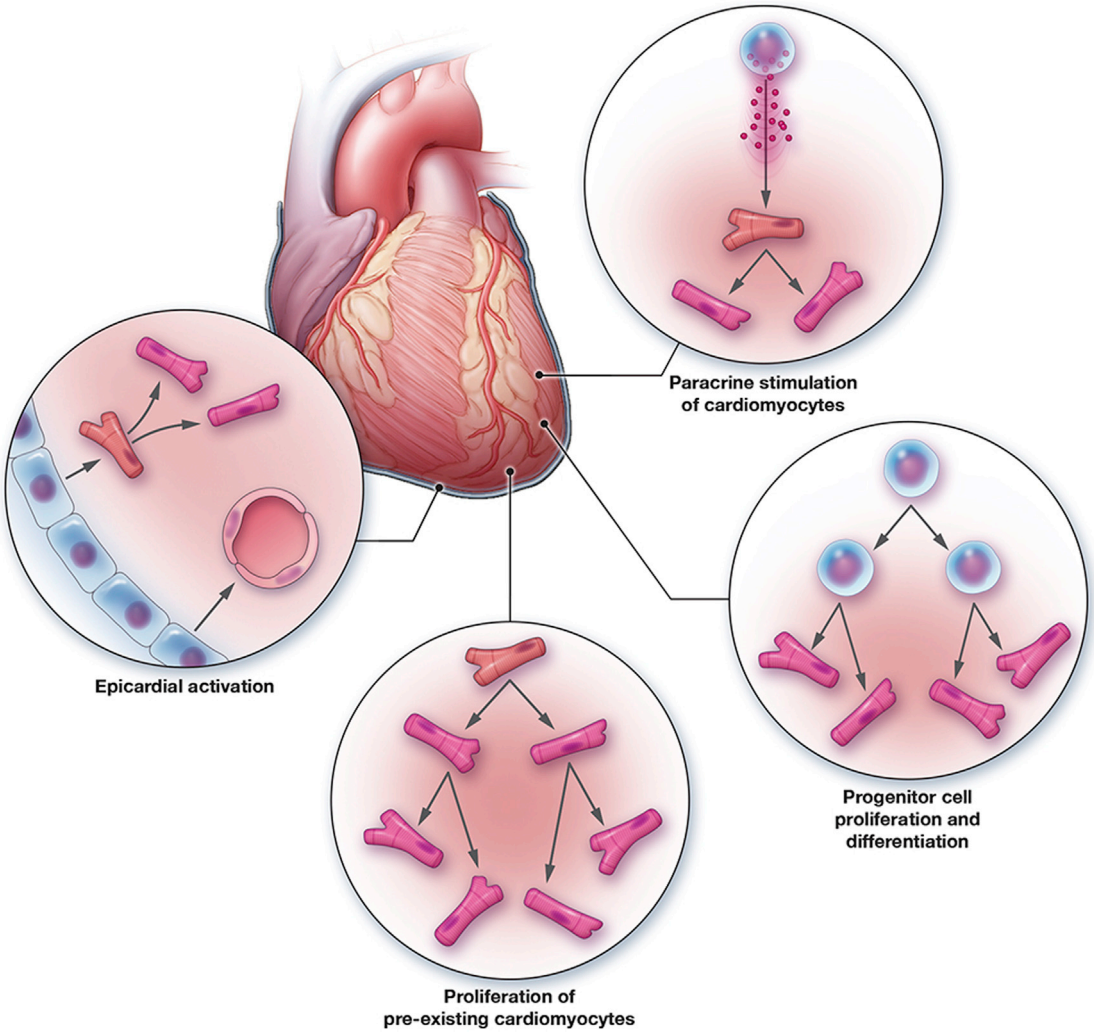

Figure 2. Proposed Mechanisms for Generation of New Cardiomyocytes after Injury
Four potential mechanisms of the heart's response to injury may lead to a regenerative response (clockwise from top): (1) paracrine factors are released by noncardiomyocyte cells to promote the proliferation of existing cardiomyocytes; (2) progenitor cells activate, proliferate, and undergo differentiation into new cardiomyocytes; (3) mature cardiomyocytes undergo dedifferentiation, reenter the cell cycle, and proliferate into new cardiomyocytes; or (4) injury results in activation of the epicardium, leading to growth of new blood vessels and/or proliferation of new cardiomyocytes.

The mechanism for cardiomyocyte homeostasis in normal mammalian myocardium is potentially different from regeneration after injury, which could trigger a cascade of signals that activate dormant progenitor cells or induce proliferation of existing cardiomyocytes (Figure 2). There is growing evidence for dedifferentiation of existing cardiomyocytes as the primary pathway for cell renewal both in injury models and during aging (Porrello et al., 2011; Senyo et al., 2013), while the magnitude of response is perhaps related to signals activated after injury.

In addition, activation of the surrounding epicardium, the thin layer of connective tissue and nonmyocytes on the outer surface of the heart, may contribute to myocardial repair after injury (Huang et al., 2012). Epicardial cells that demonstrate an epithelial-to-mesenchymal transition may lead to myocardial revascularization and perhaps to cardiomyocyte formation as well (Lepilina et al., 2006; Zhou et al., 2008a). Pretreatment of mice with thymosin beta-4 appears to enhance the formation of new cardiomyocytes derived from epicardial progenitor cells (Smart et al., 2011). However, a subsequent study in which mice were treated with thymosin beta-4 after myocardial infarction showed that injury led to epicardial activation, which resulted in angiogenesis, but not cardiogenesis (Zhou et al., 2012). Whether the epicardium in the mammalian heart is able to give rise to cardiomyocytes is a topic that remains actively discussed.

What Is the Ideal Cell Type for Cell Transplantation Approaches?

The majority of cardiac regenerative approaches in clinical trials to date have involved transplantation or infusion of cells with potential progenitor features into infarcted myocardium. Types of stem cells considered for exogenous delivery include embryonic, inducible pluripotent, and adult progenitor (including cardiac, bone marrow, and skeletal myoblast) stem cells. While there are encouraging signals of benefit in some very rigorously designed and well-performed studies, there is no consensus on

the ideal cell type to use for cell transplantation (or whether it might be advantageous to use a combination of cell types, for example, to facilitate both vasculogenesis and cardiomyogenesis). Ultimately, selection of a cell type that allows for autologous transplantation, rapid expansion in vitro, and specific differentiation into cardiomyocytes is desired.

ESCs. Since the first isolation of human ESCs in 1998 (Thomson et al., 1998), the possibility of an unlimited supply of cardiomyocytes has driven progress in deriving cardiomyocytes in vitro from human ESCs. When human ESCs are exposed to activin A and bone morphogenic protein 4, one can generate a highly purified population of human ESC-derived cardiomyocytes that, when subsequently transplanted in a prosurvival cocktail, demonstrate enhanced survival properties in vivo (Laflamme et al., 2007). Furthermore, by sorting cells based on differences in glucose and lactate metabolism, cardiomyocyte populations of up to 99% purity have been isolated from human ESC precursors (Tohyama et al., 2013). Human ESC-derived cardiomyocytes can also electromechanically couple with host cells to allow synchronous contraction between the grafted cells and the host tissue (Shiba et al., 2012). While human ESC transplantation into human myocardium has not yet been studied, teratoma formation was observed when incompletely purified human ESC-derived cardiomyocytes were transplanted into immunosuppressed Rhesus monkeys (Blin et al., 2010). Ultimately, ethical concerns may prevent the use of human ESCs for clinical cardiac regeneration; however, human ESCs remain an important laboratory tool for understanding differentiation and pluripotency in the cardiogenesis process.

Induced Pluripotent Stem Cells (iPSCs). The discovery that embryonic and mature mouse fibroblasts (Takahashi and Yamanaka, 2006) can be induced to become pluripotent stem cells by retroviral transduction of four transcription factors, OCT3/4, SOX2, c-MYC, and KLF4, revolutionized regenerative biology. Creation of iPSCs from human fibroblasts (Takahashi et al., 2007; Yu et al., 2007) heightened clinical appeal and led to rapid implementation of iPSCs as a source of cardiomyocytes (Davis et al., 2012; Nelson et al., 2009). Like ESCs, iPSCs are multipotent and clonogenic. However, iPSCs circumvent many of the ethical issues surrounding ESCs, and the ability to create autologous iPSCs from a skin biopsy, hair follicle cells, or blood (Aasen and Izpisúa Belmonte, 2010) allows potential disease modeling as well as the generation of large numbers of autologous cardiomyocytes. However, developing procedures to efficiently and cost-effectively produce sufficient quantities of autologous cells for transplantation within a therapeutic time frame remains a challenge. Different types of cardiomyocytes, including atrial-, ventricular-, and nodal-like cells, can form by differentiation of iPSCs with distributions similar to that seen with ESC-derived cardiomyocytes (Zhang et al., 2009). Alternative methods to create iPSCs that avoid the use of viral vectors have been developed to address tumorigenicity concerns (Okita et al., 2008). An important issue concerning cardiogenesis with iPSCs is achieving the long-term stability and integration into the myocardium, as many cell types derived from iPSCs are incompletely differentiated compared to the mature cell.

Skeletal Myoblasts. Skeletal myoblasts were among the first cells tested for cardiac cell therapy applications. However, the MAGIC clinical trial had disappointing efficacy results and an increased incidence of arrhythmias in patients who received intramyocardial injection of autologous skeletal myoblasts obtained via thigh muscle biopsy (Leobon et al., 2003). Because of these discouraging results, combined with the recent availability of more attractive cell sources, skeletal myoblast studies have declined in recent years.

Bone-Marrow-Derived Stem Cells. Bone-marrow-derived cells are able to differentiate in vitro into a wide variety of cells, including cardiomyocytes and vascular endothelial cells (Ohnishi et al., 2007). They can also be harvested for autologous transplantation and have shown relatively safe profiles in animal and early clinical trials (Amado et al., 2005; Hare et al., 2012). A meta-analysis of 33 randomized controlled trials studying transplantation of adult bone-marrow-derived cells to improve cardiac function after myocardial infarction revealed substantial heterogeneity between trials, but a statistically significant improvement in left ventricular ejection fraction (LVEF) in response to progenitor cell therapy that was not associated with significant improvements in morbidity or mortality (Clifford et al., 2012).

In a well-done randomized and blinded clinical trial, autologous bone marrow cells led to improved outcomes and ventricular function in patients after myocardial infarction at 2 years posttransplantation (Assmus et al., 2010) (REPAIR-AMI trial). However, two recent clinical trials evaluating the safety and efficacy of bone-marrow-derived cell therapies have been somewhat discouraging (Marbán and Malliaras, 2012). The TIME trial did not show any improvement in ventricular function after intracoronary delivery of autologous bone marrow cells (Traverse et al., 2012). Similarly, the POSEIDON trial, while demonstrating a reassuring safety profile, did not show an improvement in global ventricular function (as determined by LVEF) after transendocardial delivery of bone-marrow-derived cells in patients with ischemic cardiomyopathy (Hare et al., 2012). Whether bone marrow cells can reduce mortality after myocardial infarction is now being studied in a large multinational trial in Europe (BAMI trial).

CPCs. Many reports have described CPCs as multipotent, clonogenic cells that can differentiate into cardiomyocytes and vascular cells (Beltrami et al., 2003; Messina et al., 2004). In some publications, the presence of the c-kit marker is used as a definition of CPCs (Bearzi et al., 2007; Bolli et al., 2011). These putative progenitors can be isolated from cardiac tissue obtained during heart surgery or endocardial biopsy and then expanded in culture for use in autologous transplantation (Smith et al., 2007). The use of a single marker to isolate CPCs from adult mammalian myocardium is problematic and highly susceptible to contamination from nonprogenitor cells.

Two prominent clinical trials have reported early results after transplantation of autologous cells with human progenitor characteristics. The SCIPIO phase 1 trial demonstrated a 12.3% improvement in LVEF in patients 1 year after intracoronary injection with autologous c-kit+, lineage– CPCs following myocardial infarction (Bolli et al., 2011). In the CADUCEUS phase 1 trial, patients 2–4 weeks postmyocardial infarction were randomized to receive an intracoronary injection of cardiosphere-derived autologous stem cells or standard of care (Makkar et al., 2012). While there was no significant difference between the two groups in measures of global function, such as LVEF, there was a

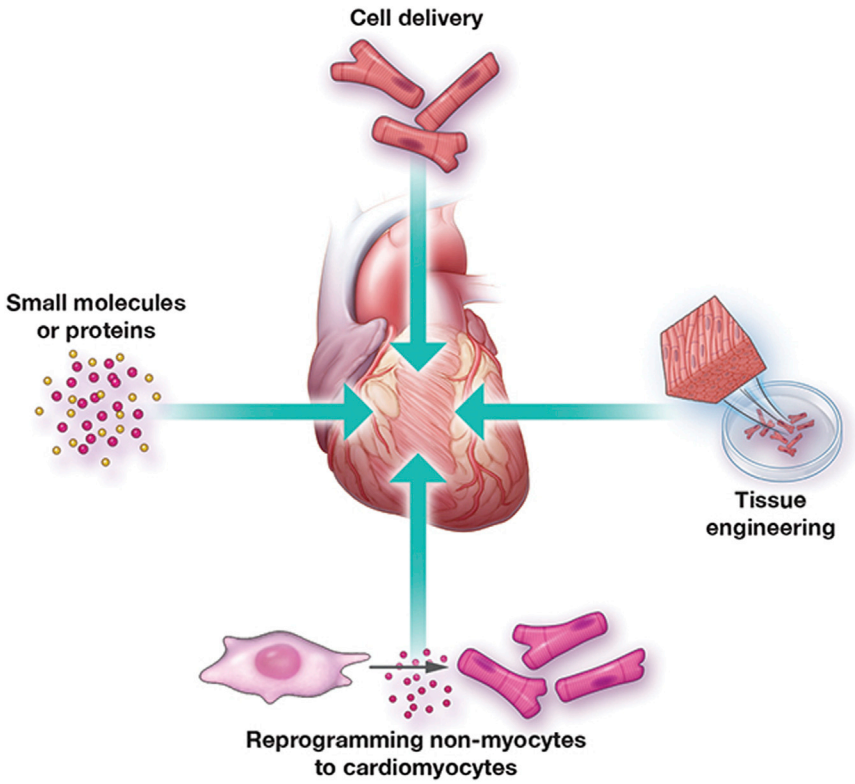

Figure 3. Approaches to Cardiac Regeneration after Injury
Multiple strategies are under investigation to promote cardiac regeneration in diseased hearts (clockwise from top): (1) cell therapy with cultured cells injected into the myocardium or coronary arteries is in clinical trials, with hopes that these cells may become functional cardiomyocytes; (2) tissue engineering approaches that combine cells with biomaterials to create functional tissue in vitro for transplantation into the heart; (3) reprogramming noncardiomyocytes into cardiomyocytes in situ may be accomplished with viruses, small molecules, or microRNAs; and (4) small molecules such as growth factors or microRNAs that are delivered to promote wound healing via cardiomyocyte proliferation or angiogenesis.

reduction of the scar mass and an increase of viable tissue and regional contractility when evaluated by cardiac magnetic resonance imaging (MRI) at 6 months (Makkar et al., 2012). No adverse events related to cell transplantation were reported in either study at 1 year (SCIPIO) or 6 months (CADUCEUS). To date, no single cell type has proven itself to meet sufficient criteria for widespread use in clinical applications, a fact that may ultimately hinder progress in cell transplantation approaches.

What Is the Mechanism of Action by which Cell Transplantation Demonstrates Clinical Efficacy?

The mechanism by which exogenous administration of autologous progenitor cells contributes to improving cardiac function remains unclear. It is possible that these autologous cells are leading to regeneration, but it is also plausible that paracrine effects or changes in the myocardial response to injury are responsible. The available technology for imaging cell fate and myocardium does not allow determination of true regeneration; therefore we must rely on surrogate measures of efficacy.

Prominent claims that bone marrow cells can become cardiomyocytes after transplantation into myocardium (Orlic et al., 2001) have not been replicated by other laboratories (Loffredo et al., 2011; Murry et al., 2004; Wagers et al., 2002). This conflict is responsible for some of the ongoing confusion in the field (Limbourg and Drexler, 2005). The use of bone marrow cells for prevention and treatment of heart failure has had varied clinical success to date but remains under intense clinical investigation as described above.

Extensive data indicate that most cells transplanted into the heart do not survive long-term, and thus the concept of paracrine effects from injected cells has become popular despite only indirect evidence for this theory (Govaert et al., 2009; Loffredo et al., 2011). In addition to the modulation of the extracellular milieu in vitro (Baffour et al., 2006), the effect of transplanted bone-marrow-derived cells on improving cardiac function may be due primarily to a paracrine effect (Gnecchi et al., 2005; Iso et al., 2007; Loffredo et al., 2011; Williams and Hare, 2011). Even in the case of human cardiosphere-derived cells, which are derived from human myocardium, the benefits of cell therapy may be paracrine (Li et al., 2012). The factors secreted or released from injected cells that benefit cardiac function remain to be identified. If there is a specific combination of multiple factors from a defined population of cells, then unraveling the paracrine cocktail may be very challenging. Furthermore, as improved methods to enhance cell survival and engraftment are developed, distinguishing between independent cell effects and paracrine effects will become even more difficult.

A major challenge in cell therapy approaches is how to improve engraftment. An excellent review by Terrovitis and colleagues describes methods to both evaluate and optimize engraftment (Terrovitis et al., 2010). Methods to quantify engraftment remain controversial, and correlation of engraftment to improvements in morbidity and mortality remain unclear. Surrogate measures of success such as global heart function with LVEF may not provide adequate resolution, although cardiac MRI may facilitate both local and global assessment. Finally, introducing cells into a hostile, diseased environment such as ischemic myocardium likely hinders engraftment, and without the reestablishment of adequate vascularization, it is unlikely that transplantation of cardiomyocytes alone will achieve success.

What Is the Ideal Approach for Clinical Cardiac Regeneration?

Multiple approaches are under investigation for human cardiac regeneration (Figure 3). As described above, significant progress has been made in cell transplantation approaches; however, these methods are challenged by poor cell survival and engraftment and may lack true regeneration. Alternatively,

reprogramming of endogenous nonmyocytes into cardiomyocytes may allow in situ transdifferentiation, although these methods require further validation before they will be ready for clinical trials.

Despite the lack of evidence for true regeneration with cell therapy approaches, clinical success will ultimately depend on evidence of clinical efficacy, and some cell therapy methods have shown limited improvement in cardiac function as described above. Importantly, cardiac cell therapy has been surprisingly safe to date. No report of tumor formation has occurred in over 1,500 patients involved in bone marrow cell cardiac trials (Clifford et al., 2012). Teratoma formation has been seen in monkeys injected with unpurified human ESC-derived cardiomyocytes (Blin et al., 2010); however, adequate purification of cardiac populations prior to transplantation may prevent tumor formation (Blin et al., 2010; Tohyama et al., 2013).

No consensus has been reached about the optimal delivery method for transplanted cells. Intravenous, intracoronary, and intramyocardial injection methods have all been proposed, although all are limited by poor local retention (Dib et al., 2011). Tissue engineering approaches combine cells with biomaterials to address logistical challenges. Use of injectable hydrogels has been studied with both natural and synthetic biomaterials to try to improve local retention (Ye et al., 2011). Biodegradable scaffolds seeded with cells can be used to form well-defined architectures as in valve tissue engineering (Schmidt et al., 2007). Finally, placement of a cardiac patch formed with stem cells can provide both structural and paracrine support after myocardial injury (Wei et al., 2008). While tissue engineering approaches are still in development, these approaches will likely augment the behavior, and ultimately the success, of transplanted cells.

Cellular reprogramming approaches aim to modify the phenotype of native cells to induce cardiomyocyte renewal via delivery of small molecules in vivo. Cellular reprogramming strategies may ultimately win over cell transplantation because of the challenges of timely production of sufficient quantities of autologous cells that meet all criteria necessary for safe and efficacious transplantation. However, much work remains before the safety and efficacy of reprogramming allows clinical testing. Aguirre and colleagues (Aguirre et al., 2013) recently provided an excellent review on animal models for cardiac reprogramming, and this topic is discussed further in the following section.

How Can We Promote Stable Differentiation of Nonmyocytes into Cardiac Phenotypes?

The possibility of skipping the multipotent state and directly reprogramming cells in vivo from one differentiated phenotype to another was demonstrated in pancreatic cells by Melton and colleagues (Zhou et al., 2008b). The Srivastava group devised a method to directly reprogram fibroblasts to cardiomyocyte-like cells using a combination of three transcription factors (GATA4, MEF2C, and TBX5) (Ieda et al., 2010). Using a retroviral system to deliver GATA4, MEF2C, and TBX5 to 2-month-old male mice in vivo via intramyocardial delivery, the same group found that cardiomyocyte-like cells were formed from the resident fibroblast population, and this intervention resulted in improved myocardial function after infarction (Qian et al., 2012). Similarly, four transcription factors (GATA4, HAND2, MEF2C, and TBX5) were used to reprogram mouse tail-tip and cardiac fibroblasts into functional cardiomyocyte-like cells in vivo (Song et al., 2012).

Subsequent studies have demonstrated direct reprogramming using microRNA (Jayawardena et al., 2012) or alternative transcription factors such as ETS2 and MESP1 (Islas et al., 2012). However, these methods exhibit low efficiency and incomplete efficacy in reprogramming fibroblasts into cardiomyocyte-like cells (Chen et al., 2012), and further investigation is required to better understand the mechanisms by which transdifferentiation occurs. If, as suggested by Srivistava and colleagues (Qian et al., 2012), maturation of reprogrammed cells can occur in vivo, then it is conceivable that long-term stable integration of reprogrammed cardiomyocytes may be possible. It remains unclear if delivery of transcription factors may have effects on noncardiac tissues in the event of poorly localized delivery, or if uncontrolled cardiomyocyte reprogramming has adverse effects such as rhythm disturbances. Prior to clinical translation of cellular reprogramming methods, we must achieve a deeper understanding of the molecular mechanisms of regeneration.

Conclusions

Stem cell biology holds significant promise for heart diseases. Because autologous cardiac cell therapy appears to be safe and possibly effective, investigators are aggressively advancing this clinical approach. At this early stage, these efforts must undergo rigorous study, preferably with randomization and blinded outcome assessment. We believe that cardiac cell therapy outside of such carefully designed and monitored trials is currently unethical. As is apparent to most investigators in the field, the current published data on cardiac regeneration and cardiac stem cells conflict in important ways. While confusion is to be expected in early days of an exciting field, this is especially true when new technologies are coming out rapidly and when clinical trials have begun, as investigators feel even more invested in the ''established'' premises underlying their work. But as the enthusiasm for cardiac regeneration charges ahead toward clinical translation, it is crucial for all investigators to maintain objectivity and seek new and complementary approaches to resolve apparent controversies.

Are we on the right path? Although it is possible that current cardiac cell therapy trials in humans are causing true regeneration, we suggest that the overall evidence is most consistent with the concept that cardiac cell therapy is regulating an endogenous repair process and not leading to true regeneration. Nonetheless, patients who achieve improved recovery will not care if we call it ''regeneration'' or ''repair,'' so enhancing heart function through cell transplantation is a worthy goal, even if it turns out not to be through true regeneration.

Ultimately, though, we must understand the dramatic differences between cardiac regeneration in experimental models like zebrafish and neonatal mice and the profound postnatal loss of cardiac regenerative potential in adult mammals like mice and humans. Is this due to intrinsic properties of cardiomyocytes or due to failure of stem/progenitor populations? Is it due to noncardiomyocytes, such as activated fibroblasts creating scarring that blocks regeneration? As in regeneration of many different mammalian organs, the core issues in cardiac regeneration remain mysterious, and we have yet to understand

what signals start the regenerative process, how regeneration is guided, and finally, how regeneration is terminated.

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REFERENCES

Aasen, T., and Izpisúa Belmonte, J.C. (2010). Isolation and cultivation of human keratinocytes from skin or plucked hair for the generation of induced pluripotent stem cells. Nat. Protoc. *5*, 371–382.

Aguirre, A., Sancho-Martinez, I., and Izpisua Belmonte, J.C. (2013). Reprogramming toward heart regeneration: stem cells and beyond. Cell Stem Cell *12*, 275–284.

Amado, L.C., Saliaris, A.P., Schuleri, K.H., St John, M., Xie, J.S., Cattaneo, S., Durand, D.J., Fitton, T., Kuang, J.Q., Stewart, G., et al. (2005). Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc. Natl. Acad. Sci. USA *102*, 11474–11479.

Assmus, B., Rolf, A., Erbs, S., Elsässer, A., Haberbosch, W., Hambrecht, R., Tillmanns, H., Yu, J., Corti, R., Mathey, D.G., et al.; REPAIR-AMI Investigators. (2010). Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circ Heart Fail *3*, 89–96.

Baffour, R., Pakala, R., Hellinga, D., Joner, M., Okubagzi, P., Epstein, S.E., and Waksman, R. (2006). Bone marrow-derived stem cell interactions with adult cardiomyocytes and skeletal myoblasts in vitro. Cardiovasc. Revasc. Med. *7*, 222–230.

Bearzi, C., Rota, M., Hosoda, T., Tillmanns, J., Nascimbene, A., De Angelis, A., Yasuzawa-Amano, S., Trofimova, I., Siggins, R.W., Lecapitaine, N., et al. (2007). Human cardiac stem cells. Proc. Natl. Acad. Sci. USA *104*, 14068–14073.

Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., et al. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell *114*, 763–776.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., et al. (2009). Evidence for cardiomyocyte renewal in humans. Science *324*, 98–102.

Blin, G., Nury, D., Stefanovic, S., Neri, T., Guillevic, O., Brinon, B., Bellamy, V., Rücker-Martin, C., Barbry, P., Bel, A., et al. (2010). A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. J. Clin. Invest. *120*, 1125–1139.

Bolli, R., Chugh, A.R., D'Amario, D., Loughran, J.H., Stoddard, M.F., Ikram, S., Beache, G.M., Wagner, S.G., Leri, A., Hosoda, T., et al. (2011). Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet *378*, 1847–1857.

Bollini, S., Smart, N., and Riley, P.R. (2011). Resident cardiac progenitor cells: at the heart of regeneration. J. Mol. Cell. Cardiol. *50*, 296–303.

Chen, J.X., Krane, M., Deutsch, M.A., Wang, L., Rav-Acha, M., Gregoire, S., Engels, M.C., Rajarajan, K., Karra, R., Abel, E.D., et al. (2012). Inefficient reprogramming of fibroblasts into cardiomyocytes using Gata4, Mef2c, and Tbx5. Circ. Res. *111*, 50–55.

Clifford, D.M., Fisher, S.A., Brunskill, S.J., Doree, C., Mathur, A., Watt, S., and Martin-Rendon, E. (2012). Stem cell treatment for acute myocardial infarction. Cochrane Database Syst. Rev. *2*, CD006536.

Davis, R.P., Casini, S., van den Berg, C.W., Hoekstra, M., Remme, C.A., Dambrot, C., Salvatori, D., Oostwaard, D.W., Wilde, A.A., Bezzina, C.R., et al. (2012). Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. Circulation *125*, 3079–3091.

Dib, N., Khawaja, H., Varner, S., McCarthy, M., and Campbell, A. (2011). Cell therapy for cardiovascular disease: a comparison of methods of delivery. J. Cardiovasc. Transl. Res. *4*, 177–181.

Dor, Y., Brown, J., Martinez, O.I., and Melton, D.A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature *429*, 41–46.

Drenckhahn, J.D., Schwarz, Q.P., Gray, S., Laskowski, A., Kiriazis, H., Ming, Z., Harvey, R.P., Du, X.J., Thorburn, D.R., and Cox, T.C. (2008). Compensatory growth of healthy cardiac cells in the presence of diseased cells restores tissue homeostasis during heart development. Dev. Cell *15*, 521–533.

Fransioli, J., Bailey, B., Gude, N.A., Cottage, C.T., Muraski, J.A., Emmanuel, G., Wu, W., Alvarez, R., Rubio, M., Ottolenghi, S., et al. (2008). Evolution of the c-kit-positive cell response to pathological challenge in the myocardium. Stem Cells *26*, 1315–1324.

Gnecchi, M., He, H., Liang, O.D., Melo, L.G., Morello, F., Mu, H., Noiseux, N., Zhang, L., Pratt, R.E., Ingwall, J.S., and Dzau, V.J. (2005). Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat. Med. *11*, 367–368.

Govaert, J.A., Swijnenburg, R.J., Schrepfer, S., Xie, X., van der Bogt, K.E., Hoyt, G., Stein, W., Ransohoff, K.J., Robbins, R.C., and Wu, J.C. (2009). Poor functional recovery after transplantation of diabetic bone marrow stem cells in ischemic myocardium. J. Heart Lung Transplant. *28*, 1158–1165, e1.

Hare, J.M., Fishman, J.E., Gerstenblith, G., DiFede Velazquez, D.L., Zambrano, J.P., Suncion, V.Y., Tracy, M., Ghersin, E., Johnston, P.V., Brinker, J.A., et al. (2012). Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA *308*, 2369–2379.

Huang, G.N., Thatcher, J.E., McAnally, J., Kong, Y., Qi, X., Tan, W., DiMaio, J.M., Amatruda, J.F., Gerard, R.D., Hill, J.A., et al. (2012). C/EBP transcription factors mediate epicardial activation during heart development and injury. Science *338*, 1599–1603.

Ieda, M., Fu, J.D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell *142*, 375–386.

Islas, J.F., Liu, Y., Weng, K.C., Robertson, M.J., Zhang, S., Prejusa, A., Harger, J., Tikhomirova, D., Chopra, M., Iyer, D., et al. (2012). Transcription factors ETS2 and MESP1 transdifferentiate human dermal fibroblasts into cardiac progenitors. Proc. Natl. Acad. Sci. USA *109*, 13016–13021.

Iso, Y., Spees, J.L., Serrano, C., Bakondi, B., Pochampally, R., Song, Y.H., Sobel, B.E., Delafontaine, P., and Prockop, D.J. (2007). Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. Biochem. Biophys. Res. Commun. *354*, 700–706.

Jayawardena, T.M., Egemnazarov, B., Finch, E.A., Zhang, L., Payne, J.A., Pandya, K., Zhang, Z., Rosenberg, P., Mirotsou, M., and Dzau, V.J. (2012). MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. Circ. Res. *110*, 1465–1473.

Jopling, C., Sleep, E., Raya, M., Martí, M., Raya, A., and Izpisúa Belmonte, J.C. (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature *464*, 606–609.

Kajstura, J., Leri, A., Finato, N., Di Loreto, C., Beltrami, C.A., and Anversa, P. (1998). Myocyte proliferation in end-stage cardiac failure in humans. Proc. Natl. Acad. Sci. USA *95*, 8801–8805.

Kajstura, J., Gurusamy, N., Ogórek, B., Goichberg, P., Clavo-Rondon, C., Hosoda, T., D'Amario, D., Bardelli, S., Beltrami, A.P., Cesselli, D., et al. (2010). Myocyte turnover in the aging human heart. Circ. Res. *107*, 1374–1386.

Kajstura, J., Rota, M., Cappetta, D., Ogórek, B., Arranto, C., Bai, Y., Ferreira-Martins, J., Signore, S., Sanada, F., Matsuda, A., et al. (2012). Cardiomyogenesis in the aging and failing human heart. Circulation *126*, 1869–1881.

Kantrowitz, A. (1998). America's first human heart transplantation: the concept, the planning, and the furor. ASAIO J. *44*, 244–252.

Kattman, S.J., Huber, T.L., and Keller, G.M. (2006). Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. Dev. Cell *11*, 723–732.

Kikuchi, K., Holdway, J.E., Werdich, A.A., Anderson, R.M., Fang, Y., Egnaczyk, G.F., Evans, T., Macrae, C.A., Stainier, D.Y., and Poss, K.D. (2010). Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. Nature *464*, 601–605.

Kouskoff, V., Lacaud, G., Schwantz, S., Fehling, H.J., and Keller, G. (2005). Sequential development of hematopoietic and cardiac mesoderm during embryonic stem cell differentiation. Proc. Natl. Acad. Sci. USA *102*, 13170–13175.

Laflamme, M.A., Chen, K.Y., Naumova, A.V., Muskheli, V., Fugate, J.A., Dupras, S.K., Reinecke, H., Xu, C., Hassanipour, M., Police, S., et al. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat. Biotechnol. *25*, 1015–1024.

Laube, F., Heister, M., Scholz, C., Borchardt, T., and Braun, T. (2006). Re-programming of newt cardiomyocytes is induced by tissue regeneration. J. Cell Sci. *119*, 4719–4729.

Leobon, B., Garcin, I., Menasche, P., Vilquin, J.T., Audinat, E., and Charpak, S. (2003). Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc. Natl. Acad. Sci. USA *100*, 7808–7811.

Lepilina, A., Coon, A.N., Kikuchi, K., Holdway, J.E., Roberts, R.W., Burns, C.G., and Poss, K.D. (2006). A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. Cell *127*, 607–619.

Li, T.S., Cheng, K., Malliaras, K., Smith, R.R., Zhang, Y., Sun, B., Matsushita, N., Blusztajn, A., Terrovitis, J., Kusuoka, H., et al. (2012). Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere-derived cells. J. Am. Coll. Cardiol. *59*, 942–953.

Limbourg, F.P., and Drexler, H. (2005). Bone marrow stem cells for myocardial infarction: effector or mediator? Circ. Res. *96*, 6–8.

Loffredo, F.S., Steinhauser, M.L., Gannon, J., and Lee, R.T. (2011). Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell *8*, 389–398.

Makkar, R.R., Smith, R.R., Cheng, K., Malliaras, K., Thomson, L.E., Berman, D., Czer, L.S., Marbán, L., Mendizabal, A., Johnston, P.V., et al. (2012). Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet *379*, 895–904.

Malliaras, K., Zhang, Y., Seinfeld, J., Galang, G., Tseliou, E., Cheng, K., Sun, B., Aminzadeh, M., and Marbán, E. (2013). Cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. EMBO Mol Med *5*, 191–209.

Marbán, E., and Malliaras, K. (2012). Mixed results for bone marrow–derived cell therapy for ischemic heart disease. JAMA *308*, 2405–2406.

Messina, E., De Angelis, L., Frati, G., Morrone, S., Chimenti, S., Fiordaliso, F., Salio, M., Battaglia, M., Latronico, M.V., Coletta, M., et al. (2004). Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ. Res. *95*, 911–921.

Mollova, M., Bersell, K., Walsh, S., Savla, J., Das, L.T., Park, S.Y., Silberstein, L.E., Dos Remedios, C.G., Graham, D., Colan, S., and Kühn, B. (2013). Cardiomyocyte proliferation contributes to heart growth in young humans. Proc. Natl. Acad. Sci. USA *110*, 1446–1451.

Moretti, A., Caron, L., Nakano, A., Lam, J.T., Bernshausen, A., Chen, Y., Qyang, Y., Bu, L., Sasaki, M., Martin-Puig, S., et al. (2006). Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. Cell *127*, 1151–1165.

Motoike, T., Markham, D.W., Rossant, J., and Sato, T.N. (2003). Evidence for novel fate of Flk1+ progenitor: contribution to muscle lineage. Genesis *35*, 153–159.

Murry, C.E., Soonpaa, M.H., Reinecke, H., Nakajima, H., Nakajima, H.O., Rubart, M., Pasumarthi, K.B., Virag, J.I., Bartelmez, S.H., Poppa, V., et al. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature *428*, 664–668.

Nelson, T.J., Martinez-Fernandez, A., Yamada, S., Perez-Terzic, C., Ikeda, Y., and Terzic, A. (2009). Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. Circulation *120*, 408–416.

Oberpriller, J.O., and Oberpriller, J.C. (1974). Response of the adult newt ventricle to injury. J. Exp. Zool. *187*, 249–253.

Oh, H., Bradfute, S.B., Gallardo, T.D., Nakamura, T., Gaussin, V., Mishina, Y., Pocius, J., Michael, L.H., Behringer, R.R., Garry, D.J., et al. (2003). Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc. Natl. Acad. Sci. USA *100*, 12313–12318.

Ohnishi, S., Ohgushi, H., Kitamura, S., and Nagaya, N. (2007). Mesenchymal stem cells for the treatment of heart failure. Int. J. Hematol. *86*, 17–21.

Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., and Yamanaka, S. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. Science *322*, 949–953.

Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S.M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D.M., et al. (2001). Bone marrow cells regenerate infarcted myocardium. Nature *410*, 701–705.

Pfister, O., Mouquet, F., Jain, M., Summer, R., Helmes, M., Fine, A., Colucci, W.S., and Liao, R. (2005). CD31- but Not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. Circ. Res. *97*, 52–61.

Porrello, E.R., Mahmoud, A.I., Simpson, E., Hill, J.A., Richardson, J.A., Olson, E.N., and Sadek, H.A. (2011). Transient regenerative potential of the neonatal mouse heart. Science *331*, 1078–1080.

Poss, K.D., Wilson, L.G., and Keating, M.T. (2002). Heart regeneration in zebrafish. Science *298*, 2188–2190.

Qian, L., Huang, Y., Spencer, C.I., Foley, A., Vedantham, V., Liu, L., Conway, S.J., Fu, J.D., and Srivastava, D. (2012). In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature *485*, 593–598.

Quaini, F., Cigola, E., Lagrasta, C., Saccani, G., Quaini, E., Rossi, C., Olivetti, G., and Anversa, P. (1994). End-stage cardiac failure in humans is coupled with the induction of proliferating cell nuclear antigen and nuclear mitotic division in ventricular myocytes. Circ. Res. *75*, 1050–1063.

Rumyantsev, P.P. (1974). Ultrastructural reorganization, DNA synthesis and mitotic division of myocytes in atria of rats with left ventricle infarction. An electron microscopic and autoradiographic study. Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. *15*, 357–378.

Schmidt, D., Achermann, J., Odermatt, B., Breymann, C., Mol, A., Genoni, M., Zund, G., and Hoerstrup, S.P. (2007). Prenatally fabricated autologous human living heart valves based on amniotic fluid derived progenitor cells as single cell source. Circulation *116*(11, Suppl), I64–I70.

Senyo, S.E., Steinhauser, M.L., Pizzimenti, C.L., Yang, V.K., Cai, L., Wang, M., Wu, T.D., Guerquin-Kern, J.L., Lechene, C.P., and Lee, R.T. (2013). Mammalian heart renewal by pre-existing cardiomyocytes. Nature *493*, 433–436.

Shiba, Y., Fernandes, S., Zhu, W.Z., Filice, D., Muskheli, V., Kim, J., Palpant, N.J., Gantz, J., Moyes, K.W., Reinecke, H., et al. (2012). Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature *489*, 322–325.

Smart, N., Bollini, S., Dubé, K.N., Vieira, J.M., Zhou, B., Davidson, S., Yellon, D., Riegler, J., Price, A.N., Lythgoe, M.F., et al. (2011). De novo cardiomyocytes from within the activated adult heart after injury. Nature *474*, 640–644.

Smith, R.R., Barile, L., Cho, H.C., Leppo, M.K., Hare, J.M., Messina, E., Giacomello, A., Abraham, M.R., and Marbán, E. (2007). Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation *115*, 896–908.

Song, K., Nam, Y.J., Luo, X., Qi, X., Tan, W., Huang, G.N., Acharya, A., Smith, C.L., Tallquist, M.D., Neilson, E.G., et al. (2012). Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature *485*, 599–604.

Soonpaa, M.H., and Field, L.J. (1997). Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. Am. J. Physiol. *272*, H220–H226.

Soonpaa, M.H., Rubart, M., and Field, and, L.J. (2013). Challenges measuring cardiomyocyte renewal. Biochim. Biophys. Acta. *1833*, 799–803.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663–676.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell *131*, 861–872.

Taussig, H.B. (1969). Heart transplantation. JAMA *207*, 951–951.

Terrovitis, J.V., Smith, R.R., and Marbán, E. (2010). Assessment and optimization of cell engraftment after transplantation into the heart. Circ. Res. *106*, 479–494.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. Science *282*, 1145–1147.

Tohyama, S., Hattori, F., Sano, M., Hishiki, T., Nagahata, Y., Matsuura, T., Hashimoto, H., Suzuki, T., Yamashita, H., Satoh, Y., et al. (2013). Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell *12*, 127–137.

Traverse, J.H., Henry, T.D., Pepine, C.J., Willerson, J.T., Zhao, D.X., Ellis, S.G., Forder, J.R., Anderson, R.D., Hatzopoulos, A.K., Penn, M.S., et al.; Cardiovascular Cell Therapy Research Network (CCTRN). (2012). Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA *308*, 2380–2389.

Wagers, A.J., Sherwood, R.I., Christensen, J.L., and Weissman, I.L. (2002). Little evidence for developmental plasticity of adult hematopoietic stem cells. Science *297*, 2256–2259.

Wei, H.J., Chen, C.H., Lee, W.Y., Chiu, I., Hwang, S.M., Lin, W.W., Huang, C.C., Yeh, Y.C., Chang, Y., and Sung, H.W. (2008). Bioengineered cardiac patch constructed from multilayered mesenchymal stem cells for myocardial repair. Biomaterials *29*, 3547–3556.

Williams, A.R., and Hare, J.M. (2011). Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ. Res. *109*, 923–940.

Wu, S.M., Fujiwara, Y., Cibulsky, S.M., Clapham, D.E., Lien, C.L., Schultheiss, T.M., and Orkin, S.H. (2006). Developmental origin of a bipotential myocardial and smooth muscle cell precursor in the mammalian heart. Cell *127*, 1137–1150.

Ye, Z., Zhou, Y., Cai, H., and Tan, W. (2011). Myocardial regeneration: Roles of stem cells and hydrogels. Adv. Drug Deliv. Rev. *63*, 688–697.

Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science *318*, 1917–1920.

Zhang, J., Wilson, G.F., Soerens, A.G., Koonce, C.H., Yu, J., Palecek, S.P., Thomson, J.A., and Kamp, T.J. (2009). Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ. Res. *104*, e30–e41.

Zhou, B., Ma, Q., Rajagopal, S., Wu, S.M., Domian, I., Rivera-Feliciano, J., Jiang, D., von Gise, A., Ikeda, S., Chien, K.R., and Pu, W.T. (2008a). Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature *454*, 109–113.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008b). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature *455*, 627–632.

Zhou, B., Honor, L.B., Ma, Q., Oh, J.H., Lin, R.Z., Melero-Martin, J.M., von Gise, A., Zhou, P., Hu, T., He, L., et al. (2012). Thymosin beta 4 treatment after myocardial infarction does not reprogram epicardial cells into cardiomyocytes. J. Mol. Cell. Cardiol. *52*, 43–47.