Heart failure is a growing epidemic caused by cardiomyocyte depletion. Current therapies prolong survival by protecting remaining cardiomyocytes but are unable to overcome the fundamental problem of regenerating lost cardiomyocytes. Several strategies for promoting heart regeneration have emerged from decades of intensive study. Although some of these strategies remain confined to basic research, others are beginning to be tested in humans. We review strategies for cardiac regeneration and summarize progress of related clinical trials.

REGENERATIVE CAPACITY OF THE HUMAN HEART

Heart failure is a growing, worldwide epidemic. Current heart failure therapies reduce heart failure morbidity and mortality by blocking chronic neurohumoral activation. However, these therapies are unable to reverse heart failure and do not address its fundamental cause, the loss of cardiomyocytes (Fig. 1). Conventional wisdom has long held that adult mammalian cardiomyocytes have exited from the cell cycle and are not added to the mature heart. However, multiple independent lines of evidence now show that new cardiomyocytes are born in the postnatal heart. Histological examination of human hearts demonstrated the existence of cardiomyocytes with mitotic figures (1). However, inferring the extent of new cardiomyocyte formation from histological data is problematic for a number of technical reasons. Chief among these are the infrequency of cardiomyocyte proliferation compared to noncardiomyocytes, the difficulty of definitively distinguishing proliferative events in cardiomyocytes versus noncardiomyocytes (2), and the tendency of cardiomyocytes to become polyploid or multinucleated, particularly in response to stress [reviewed in (3)].

The challenge of measuring cardiomyocyte proliferation has been overcome through innovative cell labeling approaches. A seminal study by Bergmann and colleagues (4) used the spike in atmospheric carbon-14 that occurred as a by-product of above-ground nuclear testing as a tracing reagent to show that human cardiomyocytes are born in the postnatal heart. They estimated that 0.5 to 1% of cardiomyocytes turn over annually, so that roughly 50% of cardiomyocytes are replenished over a human life span. Cardiomyocyte proliferation in adult mouse heart was independently confirmed using multi-isotope imaging mass spectroscopy in combination with genetic labeling of preexisting cardiomyocytes (3). This study showed that new cardiomyocytes are born from preexisting cardiomyocytes at about 0.76% per year in young adult mice. After myocardial infarction (MI), cardiomyocyte proliferation increased, as 3.2% of cardiomyocytes in the infarct border zone had undergone productive cell division over an 8-week period. Collectively, these findings overturn the long-standing axiom that the postnatal heart is nonregenerative and demonstrate new cardiomyocyte addition to the adult mammalian heart.

The existence of innate regenerative capacity in the adult mammalian heart has ignited intense interest. Augmenting or supplementing adult mammalian heart is nonregenerative and demonstrate new cardiomyocyte addition to the postnatal week. Furthermore, initial studies suggest that human cardiomyocytes continue to cycle beyond the immediate neonatal period and well into childhood (9). It will be interesting to determine whether there is indeed a protracted period of proliferative competence in infant human cardiomyocytes, and if so, how this relates to the regenerative capacity of the human heart and the current timing and strategies for repair of congenital heart disease.

In mature rodent cardiomyocytes, cell cycle genes such as cyclin A, cyclin B, and CDC2 are down-regulated, whereas cyclin-dependent kinase inhibitors are actively expressed [reviewed in (10)]. These cell cycle regulators are under transcriptional and epigenetic control in cardiomyocytes. For instance, the cell cycle inhibitors Ink4a/b are repressed by polycomb repressive complex 2 (PRC2), and inactivation of the PRC2 catalytic subunit EZH2 causes inappropriate fetal up-regulation of Ink4a/b, reduction of cardiomyocyte proliferation, and myocardial hypoplasia (11). Rb/p130-dependent heterochromatin formation at E2F-regulated cell cycle genes also stimulated cardiomyocyte cell cycle withdrawal, and inactivation of Rb and p130 led to reduced heterochromatin formation, cell cycle gene derepression, and adult cardiomyocyte cell cycle reentry (12).

Forced expression of individual cell cycle regulators has been evaluated as a means to stimulate rodent cardiomyocyte cell cycle reentry [reviewed in (10)]. In some cases, this strategy successfully stimulated cardiomyocyte proliferation but caused extensive, lethal cardiac pathol-
Fig. 1. Cardiomyocyte loss in MI. MI causes loss of ~1 billion cardiomyocytes in the adult heart. After the acute event, there is progressive fibrosis and myocyte loss in the infarct border zone, and the remaining cardiomyocytes undergo hypertrophy. Standard therapy is designed to preserve remaining cardiomyocytes, with variable efficacy.

Cardiomyocyte-specific, transgenic expression of cyclin D2 was more promising because it was not deleterious and successfully stimulated adult cardiomyocyte DNA synthesis (14). Initial infarct size after MI was no different between control and cyclin D2 transgenics, and infarct size progressively decreased over time, consistent with effective regeneration (14). These data show that overexpression of some cell cycle regulators may have salutary effects in heart disease.

Coordinated activation of promitogenic gene programs may have greater success than overexpression of individual cell cycle regulators. This goal might be achieved through the redeployment of development regulatory circuits. The YAP transcriptional coactivator is a major target of the Hippo signaling pathway, a highly conserved pathway that governs cell proliferation and organ size (15). Mutations in the Hippo/YAP pathway that enhance YAP transcriptional activity stimulated fetal cardiomyocyte proliferation and caused profound cardiac overgrowth (16–18) (Fig. 2). In the adult heart, YAP-activating mutations likewise promoted proliferation of mature cardiomyocytes, albeit less robustly than in the fetal heart, and improved myocardial recovery after MI (19, 20).

MicroRNAs (miRNAs), which, like transcription factors, also coordinate regulate multiple genes, may also offer a means to coordinate activate a mitogenic program in adult cardiomyocytes. MiRNAs are attractive as therapeutic targets [see Perspective by Olson (21)], because small RNAs or their antagonists can be efficiently delivered as synthetic small molecules or expressed in the heart using adeno-associated virus. A screen of miRNAs that enhance neonatal cardiomyocyte proliferation identified miR-590 and miR-199a, and subsequent testing showed that these stimulate adult cardiomyocyte proliferation and enhance myocardial recovery after MI in mice (22). Gain- and loss-of-function approaches also showed that the miR-17-92 cluster promotes cardiomyocyte proliferation (23). On the other hand, the miR-15 family suppresses cardiomyocyte proliferation, and its inhibition by antagonirs (sequence-specific miRNA inhibitors) stimulated cardiomyocyte proliferation and improved outcome in a murine MI model (24).

Paracrine signaling regulates developmental cardiomyocyte proliferation (Fig. 2). Activation of tyrosine kinase receptor signaling pathways through delivery of insulin-like growth factor 1 (IGF-1) activated postnatal cardiomyocyte proliferation and thus improved outcome in a murine heart failure model (25), as did FGF1 (fibroblast growth factor 1) in combination with p38 MAPK (mitogen-activated protein kinase) inhibition (26). Neuregulin (NRG1) signaling through ErbB2/ErbB4 promotes fetal cardiomyocyte proliferation and differentiation, as well as adult cardiomyocyte survival [reviewed in (27)]. NRG1 was reported to drive adult mouse cardiomyocyte proliferation (28), although this result awaits independent confirmation. As a result of its multifaceted and positive effects on heart function in animal models, recombinant human NRG1 (rhNRG1) has entered clinical trials for heart failure (Table 1). An initial clinical trial found that rhNRG1 infusion for 11 days is well tolerated in patients. The infusion acutely improved cardiac function, and the effect was sustained for up to 3 months (29). Larger, randomized, multicenter studies are under way (for example, NCT01541202).

The extent to which cardiomyocyte proliferation contributes to these salutary effects of NRG1 is currently unknown.

Overall, stimulating cardiomyocyte cell cycle reentry is an attractive strategy for cardiac regeneration because it appears to be the major endogenous mechanism for regeneration in both lower vertebrates (30, 31) and mammals (5, 8) (Fig. 2). Likely this is because it leverages endogenous, developmental mechanisms for functional heart growth. These innate developmental programs generate new, autologous cardiomyocytes and ensure their mechanical, electrical, and vascular integration into the myocardium.

Although promising, much work remains to advance these studies toward clinical translation. We must confirm that proposed interventions stimulate bona fide cardiomyocyte cell cycle reentry in the mature, adult heart, and we must place added emphasis on measuring the amount of newly generated myocardium to address the critical question of whether the proposed manipulation could achieve sufficient cardiomyocyte expansion to be therapeutically meaningful. The molecular mechanisms that limit adult cardiomyocyte proliferation remain poorly understood, and greater insights would likely enable far more robust mitogenic stimulation than presently achievable. We must show that the interventions enhance long-term myocardial outcome after myocardial insults such as MI. Finally, and perhaps most importantly, we must show that these proposed treatments are safe and not detrimental to the heart or other organs. A major concern for pro-proliferative therapies is their oncogenic potential. Strategies to selectively target cardiomyocytes are needed, and mitogenic therapies will need to be rigorously evaluated for oncogenesis before they can be translated to the clinical setting.
Cardiomyocyte cell cycle reentry

**Advantages**
- Autologous
- No need to stimulate CM differentiation and maturation
- New CMs likely to have excellent mechanical, vascular, and electrical integration with host myocardium

**Challenges**
- Potential oncogenesis and other off-target effects
- Achieving sufficient magnitude of new cardiomyocyte formation.
- Genetic manipulations require gene therapy approaches; nongenetic approaches (e.g., paracrine factors) desirable

**Fig. 2. Cardiac regeneration through stimulation of adult cell cycle reentry.** Existing cardiomyocytes proliferate to replenish lost cardiomyocytes. Cardiomyocyte refreshment through stimulation of endogenous pathways may allow newly formed cardiomyocytes to integrate and recruit their own vasculature.

**STRATEGY 2: CELL THERAPY**

Transplantation of many different cell types has been proposed as a means to augment cardiac repair and regeneration (Fig. 3). Early work considered transplantation of differentiated cells such as skeletal myoblasts. It was hoped that these muscle cell progenitors would differentiate into cardiac muscle, but they did not; rather, the grafts formed myotubes that did not electrically integrate with host myocardium. Nevertheless, functional improvement by skeletal myoblasts in preclinical studies motivated their testing in patients. A number of clinical trials collectively suggested limited efficacy and elevated arrhythmia risk, perhaps as a result of poor electrical integration [reviewed in (32)]. Although disappointing, this experience laid a critical foundation for subsequent work in cardiac cell therapy and underscored the need for effective graft integration to avoid arrhythmogenesis.

More recent work has focused on progenitor cells with cardiomyogenic activity or on their differentiated derivatives. These cells can be classified into those originating from pluripotent stem cells [embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs)] (Fig. 3A) and those arising from adult progenitor cells located in the heart (resident CPCs) or in noncardiac sites (nonresident CPCs) (Fig. 3B).

**Pluripotent stem cells**

ESCs have a nearly unlimited capacity for self-renewal and can be efficiently differentiated into cardiomyocytes (33). Undifferentiated ESCs formed teratomas when injected into immunocompatible host hearts (34), a complication that will need to be excluded in pluripotent cell–based therapies. Injection of differentiated, murine ESC–derived cardiomyocytes into immunocompatible hearts yielded stable intracardiac grafts that improved cardiac function after MI (35). Similarly, human ESC–derived cardiomyocytes formed stable grafts that improved function of infarcted rat heart (36), and their electrical integration with host myocardium was recently demonstrated in a guinea pig myocardial injury model (37). Co-delivery of paracrine factor cocktails and bioengineered microenvironments enhanced survival, vascularization, and maturation of ESC-derived cardiomyocytes (36, 38). Proof of principle that this strategy could achieve clinical scale remuscularization was recently achieved by demonstrating that human ESC–derived cardiomyocytes form extensive myocardial grafts supplied by host vessels in a nonhuman primate ischemic myocardial injury model (39). The graft electrically coupled with host myocardium, but nonfatal ventricular arrhythmias were noted.

Transplantation of human ESC–derived cardiomyocytes may be therapeutically viable in people. However, aside from ethical considerations, there are several challenges and limitations that need to be considered. One fundamental challenge is the relative immaturity of current ESC-derived cardiomyocytes. Although these cells contract and generate force, their immaturity likely reduces their efficacy and host integration. ESC-based therapies will require immunosuppression to avoid graft rejection. Finally, the safety (lack of teratoma formation or arrhythmogenesis) and longevity of ESC-based grafts will need to be carefully demonstrated.

In most respects, iPSCs behave like ESCs, and thus offer their conceptual advantages. At the same time, iPSCs sidestep the ethical issues that surround ESCs. Because it is possible to generate autologous iPSCs, iPSCs would also circumvent the need for immunosuppression. However, production of such cells will require months of preparation, precluding their deployment for acute or subacute illnesses such as MI. Furthermore, the uniform manufacture of iPSC-derived cardiomyocytes from individual patients is a major logistical and regulatory hurdle for clinical use of iPSC-derived cells.

**Resident CPCs**

Several resident CPCs have been reported, including c-kit⁺ CPCs, Is11⁺ CPCs, Sca1⁺ CPCs, side-population CPCs, and cardiosphere-derived cells (CDCs). The interrelationships and overlaps between populations isolated by different groups are often uncertain, posing a hurdle to integrating the diverse studies in this area. Here, we will focus on c-kit⁺ CPCs and CDCs as illustrative examples of the potential benefits, challenges, and controversies associated with this therapeutic strategy.

The receptor tyrosine kinase c-kit (CD117) is highly expressed on hematopoietic stem cells, as well as mature circulating cells, mast cells, some endothelial cells, and immature cardiomyocytes (40). The Anversa group reported that c-kit⁺ cells isolated from the heart are self-renewing, clonogenic, and multipotent, capable of differentiating into cardiomyocytes, smooth muscle cells, and endothelial cells in vitro (41). When injected into injured rodent hearts, c-kit⁺ CPCs formed functional blood vessels and cardiomyocytes in the newly regenerated myocardium (41, 42), and indeed were found to be necessary and sufficient for cardiomyocyte regeneration and myocardial repair (43).
The cardiogenic activity of c-kit+ CPCs is highly controversial. Most c-kit+ cells in the heart originate from the bone marrow (44), and other groups reported that c-kit+ CPCs from adult mouse heart lack cardiomyogenic potential (40, 45). However, these groups did report cardiomyogenic activity in neonatal mouse c-kit+ CPCs, suggesting that the cardiogenic activity of c-kit+ cardiac cells may be developmentally regulated. Concerns over data integrity led to the recent retraction of a prominent manuscript on cardiac renewal (46), raising further questions on studies of c-kit+ CPC cardiogenic activity from these same investigators.

On the basis of preclinical studies demonstrating the beneficial effects of c-kit+ CPCs on myocardial outcome after MI, these cells were tested in humans with ischemic heart failure who underwent coronary bypass surgery. The results of these clinical trials are summarized in Table 1.

Table 1. Clinical trials in cardiac regeneration. This table summarizes the larger trials that have been performed for human heart regeneration. Additionally, a comprehensive review of reported trials can be found in (31). Outcomes listed reached statistical significance within the study. CABG, coronary artery bypass grafting; BMC, bone marrow cell; PBC, peripheral blood mononuclear cell; Std, standard therapy (no placebo); EF, ejection fraction; LV, left ventricle; CMP, cardiomyopathy.

<table>
<thead>
<tr>
<th>Trial name/phase</th>
<th>Reagent</th>
<th>Delivery method and timing</th>
<th>Patient n (intervention/control)</th>
<th>Indication</th>
<th>Follow-up</th>
<th>Outcome</th>
<th>Design</th>
<th>Ref.</th>
<th>Clinical Trials Registry</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRG1/phase 1</td>
<td>rhNRG1</td>
<td>Intravenous infusion for 11 days</td>
<td>15/baseline</td>
<td>Stable chronic heart failure</td>
<td>3 months</td>
<td>EF ↑3.9% versus baseline</td>
<td>Nonrandomized, open-label</td>
<td>(29)</td>
<td>ACTRN12607000330448*</td>
</tr>
<tr>
<td>MAGIC/phase 2</td>
<td>Skeletal muscle myoblast</td>
<td>Myocardial injection / during CABG surgery</td>
<td>63/34</td>
<td>Heart failure (EF &lt;35%)</td>
<td>6 months</td>
<td>EF unchanged</td>
<td>Multicenter, randomized, double-blind</td>
<td>(104)</td>
<td>NCT00102128†</td>
</tr>
<tr>
<td>SCIPO/phase 1</td>
<td>c-kit+ CPCs</td>
<td>Intracoronary injection/113 days after CABG</td>
<td>16/7</td>
<td>Ischemic CMP</td>
<td>1 year</td>
<td>EF% ↑12.3%*</td>
<td>Randomized, open-label</td>
<td>(47)</td>
<td>NCT00474461†</td>
</tr>
<tr>
<td>CADUCEUS/phase 1</td>
<td>CDCs</td>
<td>Intracoronary injection /1.5 to 3 months after MI</td>
<td>17/8</td>
<td>Acute MI</td>
<td>1 year</td>
<td>EF unchanged</td>
<td>Randomized, open-label</td>
<td>(52)</td>
<td>NCT00893360†</td>
</tr>
<tr>
<td>REPAIR-AMI/phase 2</td>
<td>BMCs</td>
<td>Intracoronary injection/3 to 7 days after MI</td>
<td>101/103</td>
<td>Acute MI</td>
<td>2 years</td>
<td>EF unchanged</td>
<td>Randomized, double-blind</td>
<td>(105)</td>
<td>NCT00279175†</td>
</tr>
<tr>
<td>HEBE/phase 2</td>
<td>BMCs</td>
<td>Intracoronary injection/3 to 8 days after MI</td>
<td>69/66/65 BMC/PBC/Std</td>
<td>Acute MI</td>
<td>4 months</td>
<td>EF unchanged</td>
<td>Randomized, open-label</td>
<td>(66)</td>
<td>ISRCTN95796863‡</td>
</tr>
<tr>
<td>LateTIME/phase 2</td>
<td>BMCs</td>
<td>Intracoronary injection/14 to 21 days after MI</td>
<td>55/26</td>
<td>Acute MI</td>
<td>6 months</td>
<td>EF unchanged</td>
<td>Randomized, double-blind</td>
<td>(67)</td>
<td>NCT00684060†</td>
</tr>
<tr>
<td>Jannsens/phase 2</td>
<td>BMCs</td>
<td>Intracoronary injection/1 day post MI</td>
<td>33/34</td>
<td>Acute MI</td>
<td>4 months</td>
<td>EF unchanged</td>
<td>Randomized, double-blind</td>
<td>(65)</td>
<td>NCT00264316†</td>
</tr>
<tr>
<td>BM-MNC/phase 2</td>
<td>BMCs</td>
<td>Intracoronary injection/5 to 7 days or 3 to 4 weeks after MI</td>
<td>133/67</td>
<td>Acute MI</td>
<td>4 months</td>
<td>EF unchanged</td>
<td>Open-label</td>
<td>(68)</td>
<td>NCT00355186</td>
</tr>
</tbody>
</table>

*Australian New Zealand Clinical Trials Registry. †ClinicalTrials.gov. ‡International Standard Randomised Controlled Trial Register.

The cardiogenic activity of c-kit+ CPCs is highly controversial. Most c-kit+ cells in the heart originate from the bone marrow (44), and other groups reported that c-kit+ CPCs from adult mouse heart lack cardiomyogenic potential (40, 45). However, these groups did report cardiomyogenic activity in neonatal mouse c-kit+ CPCs, suggesting that the cardiogenic activity of c-kit+ cardiac cells may be developmentally regulated. Concerns over data integrity led to the recent retraction of a prominent manuscript on cardiac renewal (46), raising further questions on studies of c-kit+ CPC cardiogenic activity from these same investigators.

On the basis of preclinical studies demonstrating the beneficial effects of c-kit+ CPCs on myocardial outcome after MI, these cells were tested in humans with ischemic heart failure who underwent coronary bypass surgery. The results of these clinical trials are summarized in Table 1.
artery bypass graft surgery in a randomized, open-label, phase 1 study called SCIPIO (Table 1). Four months after surgery, autologous c-kit+ CPCs, expanded from myocardial tissue harvested during surgery, were administered by intracoronary infusion (47). No adverse events related to CPC treatment were noted. CPC-treated patients had slight, statistically significant improvement in the left ventricular ejection fraction compared to untreated controls at 4 months (36% versus 29%). The difference was sustained in interim analysis at 2 years (48). This beneficial effect is unlikely to arise from c-kit+ CPC cardiomyogenic activity, but rather may arise through paracrine effects (see “Mechanism of action”).

CDCs are the second resident CPC population that has been intensively studied. Primary cardiospheres are phase-bright cell clusters isolated from myocardial explants by their spontaneous detachment from the growth surface (49). Replated cardiospheres expand to yield CDCs. CDCs are self-renewing, clonogenic, and multipotent, differentiating into cardiomyocytes, smooth muscle cells, and endothelial cells (50). Cardiac CDC delivery reduced scar size and improved heart function in rodent and porcine models of ischemic and nonischemic heart disease (49). Injected CDCs survived in host myocardium up to 20 days after MI, and some cells differentiated into cardiomyocytes. CDCs also function by secreting paracrine factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and IGF-1 (51).

The salutary effects of CDCs in preclinical models led to a phase 1 clinical trial where autologous CDCs, expanded from endomyocardial biopsy specimens, were delivered by intracoronary infusion 1.5 to 3 months after MI (CADUCEUS; Table 1). No adverse events related to CDC delivery were detected. CDC-treated patients had reduced scar mass and regional contractile dysfunction but no changes in global measures of left ventricular function compared to patients who required standard therapy with up to 1 year of follow-up (52, 53).

Nonresident CPCs

CPCs do not necessarily reside in the heart. In mice transplanted with LacZ-expressing bone marrow, LacZ+ cardiomyocytes and endothelial cells appeared in the heart at a low frequency (0.02 and 3%, respectively) (54). In humans, female hearts transplanted into male recipients contained XY cardiomyocytes, although the extent of chimerism varied widely in different studies (0.04 to 10%) (55–57). Hearts of sex-mismatched bone marrow transplant patients also contained cardiomyocytes with donor genetic material, pointing to bone marrow as a source of these cells.

**Fig. 3. Cardiac regeneration through cell therapy.** (A) ESC- or iPSC-derived cardiomyocytes are produced in vitro and delivered to the myocardium. (B) Resident and nonresident cardiac progenitors are isolated, expanded in vitro, and delivered to the myocardium.
source of nonresident CPCs capable of de novo cardiomyocyte formation in humans (58).

Anversa and colleagues tested hematopoietic lineage-negative, c-kit+ bone marrow cells for myocardial repair. They reported that injection of these bone marrow cells into the infarct border zone in mice formed new myocardium that occupied 68% of the infarcted portion of the ventricle (59). However, the differentiation of transplanted c-kit+ bone marrow cells into cardiomyocytes could not be reproduced by two other groups (60, 61). Fusion of transplanted cells with host cardiomyocytes, microscopy artifacts, and data selection may be the reasons why the groups reached such divergent conclusions.

Nevertheless, enthusiasm for cardiac regeneration from an autologous source as accessible as the bone marrow led to a number of clinical trials in patients with ischemic and nonischemic heart disease [reviewed in (32)]. These trials have tested unfractonated bone marrow mononuclear cells or fractionated subsets, primarily mesenchymal stromal cells, hematopoietic stem cells, and endothelial progenitor cells. These trials established that bone marrow–derived cell preparations can be safely delivered via direct intramyocardial or intracoronary injection. Administration of these cells had, at most, modest intermediate-term benefit in some studies (62–65) and no benefit in others (66–68) (Table 1). Additional phase 1/2 clinical trials are under way to further assess the efficacy of bone marrow–derived progenitor cells in heart failure (for example, NCT01569178 and NCT01781390). Overall, these data from numerous groups have tempered the initial excitement that bone marrow cells might drive robust cardiac regeneration.

**Mechanism of action**

How does cell therapy enhance outcome in heart disease? Most forms of cell therapy were initiated with the goal of replenishing lost cardiomyocytes through differentiation of injected cells into cardiomyocytes (Fig. 3). Pluripotent stem cell–derived cardiomyocytes do form stable myocardial grafts that likely functionally contribute to contractile force generation. However, most injected adult progenitor cells fail to efficiently engraft in the heart. For example, after direct intramyocardial injection of c-kit+ CPCs into infarcted mouse heart, 25% of cells present at 5 min remained at 24 hours, 7.6% remained at 7 days, and 2.8% remained at 35 days (69). Similarly, low engraftment rates were found for CDCs (51) and bone marrow–derived progenitors (70). Engraftment is even lower after intracoronary injection compared to intramyocardial injection (71). Considering the number of cells delivered to patients (millions to hundreds of millions), the low rate of engraftment, the cardiomyocyte deficit (hundreds of millions), and the low frequency of progenitor cell–derived cardiomyocytes in most studies, it is clear that the number of cardiomyocytes formed directly from injected cells is too low to account for their reported functional benefits.

These observations led to the hypothesis that transplanted progenitor cells secrete paracrine factors that promote cardiac repair and regeneration (72). Adult stem cells release a host of soluble factors [reviewed in (72)], which may act on cardiomyocytes to enhance survival (73–75) and augment proliferation (76). Paracrine factors secreted by injected progenitor cells may also act on noncardiomyocytes to promote myocardial neovascularization (73, 77–79), increase activity of resident cardiac stem cells (76, 80), and favorably modify the extracellular matrix (73, 81). Indeed, progenitor cell–conditioned media injection itself enhanced outcome after MI in some animal studies (75, 79, 82, 83). Further efforts are required to understand the beneficial actions of injected progenitor cells and to pinpoint the responsible paracrine factor(s).

In summary, there has been tremendous excitement about the potential regenerative activity of cardiac progenitors. For pluripotent stem cell–derived cardiomyocytes, the challenge will be to obtain adequate quantities of mature cardiomyocytes, to effectively integrate them with endogenous myocardium, and to ensure that these therapeutic goals can be reached with minimal risk of teratoma formation. iPSCs offer a means to achieve immunocompatibility and to sidestep ethical concerns linked to ESCs, but logistical and regulatory barriers to their clinical deployment will need to be overcome. Thousands of heart failure patients have already received adult progenitor cell therapy. Adult progenitor cells can be delivered safely, at least at current low engraftment rates, but efficacy has been modest at best and inconsistent between studies. However, these are still early days, and a number of critical questions need to be answered before the ultimate therapeutic potential of CPC therapy will be known. The optimal cell type, delivery route, dose, and frequency will need to be determined. Cell engraftment will need to be markedly improved.

Much more needs to be learned about the mechanisms of action of injected progenitor cells, so that their beneficial effects can be amplified and refined. If the major activity of injected cell comes from elaboration of paracrine factors, then focused efforts on defining and delivering the optimal paracrine factor cocktails may circumvent many of the logistical hurdles of cell therapy and thereby accelerate therapeutic translation (see following section).

![Fig. 4. Modulating resident cardiac progenitor activity in situ.](https://www.sciencemag.org/content/sci/345/6197/1174/F1.large.jpg)
STRATEGY 3: ENHANCING THE ACTIVITY OF ENDOGENOUS CPCs

Cell therapy seeks to isolate, amplify, differentiate, and then transplant progenitor cells or their progeny. An alternative strategy is to enhance the regenerative activity of resident CPCs in situ (Fig. 4). Current data indicate that cardiac progenitors do not substantially differentiate into cardiomyocytes during normal murine heart homeostasis. However, myocardial injury stimulated increased birth of cardiomyocytes from nonmyocytes (84). Pharmacological or genetic augmentation of this process may enhance cardiac repair and regeneration.

An early study in this vein showed that c-kit+ CPCs express receptors for HGF and IGF-1 (85). HGF acted as a chemotactic factor that enhanced the migration of c-kit+ cells toward the injection site, whereas IGF-1 increased the survival and proliferation of the cells. The combination of HGF and IGF-1 thus mobilized and amplified resident c-kit+ CPCs and improved myocardial outcome after MI.

Another cardiac progenitor population is located within the epicardium, a specialized epithelial sheet that covers the heart (86). Epicardial cells play a central role in heart development by undergoing epithelial-to-mesenchymal transition to form epicardium-derived cells (EPDCs), multipotent cells that differentiate into most cardiac fibroblasts and coronary smooth muscle cells. EPDCs also contribute less frequently to the endothelial and cardiomyocyte lineages (87, 88). As a result of the multiple roles of epicardium in cardiac development (86), the partial restoration of these roles in heart injury (79), and its accessibility on the surface of the heart, there has been great interest in modulating epicardial function to enhance myocardial repair and regeneration. Although, normally, epicardium does not contribute to the cardiomyocyte lineage of the adult heart (79), systemic treatment with the peptide thymosin β4 from 7 days before 14 days after LAD (left anterior descending coronary artery) ligation resulted in de novo generation of cardiomyocytes from epicardium (89). Notably, this switch in epicardial fate was not observed in the absence of MI, nor was it observed when thymosin β4 “priming” before the MI was omitted (90). Although the number of cardiomyocytes formed through this mechanism (6.6% of peri-infarct cardiomyocytes) was too low to substantively enhance myocardial function (89), this study established the principle that paracrine secreted factors modulate epicardial progenitor cardiogenic activity after MI.

Paracrine factor efficacy often depends on precise regulation of dose, location, timing, and duration. Standard methods of administration using peptides, DNA plasmids, or viral vectors may not have the pharmacodynamic profiles required for optimal paracrine factor activity. In collaboration with Zangi and colleagues (91), we explored the use of “modified RNA (modRNA)” as an alternative delivery method with unique kinetics. modRNA was synthesized by in vitro transcription in the presence of substituted nucleotides efficiently transfects many primary cell types, including cardiac cells, but avoids innate immune system activation and cytotoxicity. modRNA achieves “pulse-like” kinetics, with maximal expression at ~18 hours and minimal residual expression by 72 hours. VEGF modRNA injected into the murine heart at the time of MI reduced infarct size and enhanced neovascularization, leading to improved heart function at 3 weeks and significantly improved survival for over 1 year. In contrast, VEGF delivered by DNA plasmid injection at the time of MI reduced survival compared to controls, likely owing to sustained VEGF expression that promoted the formation of excessively permeable vessels. VEGF modRNA markedly amplified EPDCs and mobilized them so that they actively penetrated the myocardium. Moreover, VEGF redirected EPDC differentiation away from the smooth muscle and fibroblast lineages and toward the endothelial lineage, thereby enhancing perfusion of the infarct border zone.

The cell-free regenerative paradigm advanced by this VEGF modRNA study, in which paracrine signals delivered at the right time and place modulate progenitor cell activity, and its implementation using modRNA will likely apply to cardiogenic activity of CPCs (Fig. 4). For example, the Notch signaling pathway reportedly stimulates differentiation of c-kit+ CPCs toward the myocyte lineage, but maintains the newly formed cells in a proliferative state (92). Transgenic overexpression of the Notch ligand Jagged1 on cardiomyocytes reduced cardiac fibrosis and promoted cardiac precursor expansion (93).

Given the cost and logistical difficulties in translating cell-based progenitor therapy, in situ enhancement of cardiac progenitor activity is...
certainly an attractive strategy (Fig. 4). Further work will need to establish the safety of mRNA in humans and to define a paracrine factor cocktail that robustly stimulates cardiac regeneration when administered in a clinically realistic dosing regimen.

**STRATEGY 4: DIRECT REPROGRAMMING OF NONCARDIOMYOCYTES**

The discovery that expression of MyoD in fibroblasts stimulated their transdifferentiation into skeletal muscle established the paradigm of cellular reprogramming. This paradigm has been expanded in recent years in the wake of Takahashi and Yamanaka's startling discovery that fibroblasts can be transcriptionally reprogrammed to pluripotency (94). Subsequently, conversion of one different differentiated cell type to another without proceeding through a pluripotent intermediate, known as direct reprogramming, was reported for several cell types, including cardiomyocytes (Fig. 5). Retroviral delivery of Tbx5, MeF2c, and Gata4, three central cardiac transcription factor genes, successfully directs reprogramming of murine fibroblasts into "induced cardiomyocyte-like cells" (iCMs) (95). Subsequent studies demonstrated fibroblast-to-cardiomyocyte reprogramming in vivo in the context of MI, associated with reduced infarct size and improved cardiac function (96). Genetic lineage tracing suggested that directly reprogramming generated roughly one-third of cardiomyocytes in the infarct border zone: 38 to 58% of iCMs had mature sarcomere structure by immunofluorescence staining, whereas the remaining iCMs appeared less mature. Similar findings were concurrently reported for adult mouse tail-tip and cardiac fibroblasts (97). Other groups have subsequently reported fibroblast conversion to cardiomyocytes using alternative sets of reprogramming factors (98, 99) and extended these results to human fibroblasts (100–103). Together, these studies provide proof of concept that fibroblasts can be directly reprogrammed into cardiomyocytes.

Direct reprogramming of cardiac fibroblasts to iCMs raises the tantalizing possibility that the same cells that cause deleterious scarring in MI can be repurposed in situ to replenish lost cardiomyocytes (Fig. 5). The approach would obviate many of the difficulties faced by progenitor cell therapy, such as immunocompatibility, manufacture and delivery of a large number of cells, and achieving stable engraftment, differentiation, and functional integration. However, this approach comes with a new set of hurdles. Confirming fibroblast-to-iCM reprogramming in vivo currently relies on genetic lineage tracing approaches, which is not applicable to humans and have numerous caveats and pitfalls, such as cell fusion and imperfect regulation of Cre activity, which could potentially result in erroneous classification of a cardiomyocyte as having arisen from reprogramming. Thus, we need independent approaches to quantitate direct reprogramming in vivo.

The efficiency of reprogramming and the maturity and function of iCMs need to be improved. Mechanical and electrical integration of reprogrammed cardiomyocytes needs to be further assessed, and it will be important to determine whether reprogramming has proarrhythmic consequences, for instance, owing to the presence of less mature or poorly integrated iCMs. It appears that human cells require different sets of reprogramming factors than mouse cells (100–102); if true, then appropriate animal models will need to be developed for preclinical testing of cardiac reprogramming protocols targeted for use in humans. At present, all direct reprogramming approaches have used integrating viruses, which are associated with risk of oncogenesis and other consequences of genomic disruption. Development of optimized and preferably non-integrating methods for direct reprogramming will be essential to begin clinical translation of this promising regenerative strategy.

**SUMMARY AND COMMENTS**

The past decade has seen a sea of change in cardiac biology. Rather than viewing the heart as a terminally differentiated organ devoid of regenerative capacity, the mainstream view has shifted to accept that the heart has intrinsic—albeit inadequate—regenerative mechanisms. This conceptual reorientation has opened the door to innovative approaches to augment or supplement these innate regenerative abilities. As a result, there are now multiple strategies for cardiac regeneration under active development, each with its own advantages and challenges for translation. Time will tell which of the above strategies, or combination of strategies, will achieve robust cardiac regeneration in humans.

**REFERENCES AND NOTES**


