Stem Cells 1

Towards regenerative therapy for cardiac disease

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Development of regenerative therapeutic strategies to reverse the progression of advanced heart failure is one of the most urgent clinical needs of this century. Insights gained from clinical trials of adult stem cells, together with fundamental scientific advances in cardiac stem cell and regenerative biology, are beginning to yield potential new targets and strategies for regenerative therapies. Of particular importance are new scientific discoveries related to intrinsic cardiac regeneration, renewal factors that can trigger regeneration, and tissue-engineering technology, which are beginning to change the way investigators view the scientific and clinical position of cardiovascular regenerative therapy.

Introduction

Heart failure is one of the key causes of morbidity and mortality worldwide.1 The effects of heart failure are growing very rapidly, especially in developing countries.2 Available medical and device-based therapies can ameliorate the effects of heart failure, but cannot reverse the loss of functional myocardium, which is the underlying cause of the problem.3 The only available cure for advanced heart failure is orthotopic heart transplantation, which is not a viable strategy in the general population because of a relative absence of donor hearts.4 Heart failure is thus evolving into a global epidemic for which medicine does not have an available solution. Design of an efficacious regenerative therapeutic strategy has therefore become a holy grail of modern cardiovascular science. Recent breakthroughs in stem cell biology provide large strides toward this goal. This report discusses key opportunities and challenges in movement from academic stem cell biology towards the development of cardiovascular regenerative therapy.

Attempted cardiac regeneration with non-cardiac progenitor cells

The severity of heart failure and the inability of available treatments to abrogate its effects have spurred intense interest in cardiac regeneration. Several organs, notably the liver, have clinically relevant regeneration after injury.5 Although cardiac regeneration is reported in lower vertebrates such as amphibians and zebrafish, equivalent regenerative capacity is not possessed by adult mammals.6 Cardiomyocyte proliferation is present in neonatal mice, but diminishes rapidly after birth,8 and dividing cardiomyocytes are rare in the hearts of adult human beings.9,10 Cardiomyocyte turnover rate is about 1% per year in young adults, and decreases to 0·5% per year in elderly individuals.8 Turnover increases in response to injury, and higher numbers of immature, dividing cardiomyocytes are noted at infarct borders;12 however, the clinical course of heart failure shows that this pool of dividing cells cannot independently reverse the effects of a large insult.13 When development of cell-based regenerative therapies for heart failure was first started, the belief was that cardiac cell populations with regenerative potential, such as dividing cardiomyocytes and cardiac progenitors, were too rare and difficult to isolate to be of practical use. Motivated by the magnitude of the clinical issues that might be solved with cardiac regenerative therapy, several investigators chose to assess the regenerative capacity of non-cardiac progenitor cells rather than wait for characterisation of cardiac progenitors. This approach was based on the assertion that non-cardiac progenitor cells in the mesodermal lineage have sufficient developmental plasticity to differentiate into cardiac cells if situated in the appropriate niche.14 A myocardial infarction can lead to loss of up to a billion cardiomyocytes, or about 25% of myocardial mass. Skeletal myoblasts, bone marrow cells, and peripheral blood stem cells were among the first cell types investigated for cardiac regeneration, because they were plentiful and comparatively well characterised (figure 1). The first studies in animals involving transplantation of skeletal myoblasts into infarcted myocardium showed improvement in cardiac function after transplantation. This finding added credibility to assertions about the plasticity of non-cardiac progenitors.15 The mechanism by which these cells produced the reported benefit was not understood, but these data led to initiation of studies in human beings involving myoblasts, including the
Non-cardiac progenitors

Key clinical issues:
- Limited durability, maturation of cells in situ
  - Ideal progenitor identified?
- Complex three-dimensional structures not recreated
  - Recreation of three-dimensional cardiac structures?
- Arrhythmias noted after cell injection
  - Electromechanical dissociation of engrafted cells?
- Limited cell survival
  - Tissue milieu supportive of cell growth?
  - Immune rejection?
- Limited delivery of cells to target area, haematogenous dissemination of cells
  - Efficiency of the delivery system sufficient?
- Scalability
  - Clinically relevant number of therapeutic cells available?

Cardiac cells and putative cardiac progenitors

- Attempted identification of an ideal cardiac progenitor cell population
- Revascularisation
- Improvement of delivery technology

Figure 1: Milestones in clinical trials of cardiac regenerative therapies

Early clinical trials aimed to discover a candidate cardiac regenerative therapy from readily available populations of non-cardiac progenitor cells. Modest improvements in cardiac function after myocardial infarction were reported with these cells, but improved knowledge of cardiac progenitor cells and the advent of induced pluripotent stem cell technology allowed investigation of the rarer cardiac cells with regenerative potential, including putative progenitor cells; however, clinical validation is needed. G-CSF = granulocyte colony-stimulating factor.
multicentre, randomised myoblast autologous grafting in ischaemic cardiomyopathy (MAGIC) trial;26 however, studies of skeletal myoblasts in animals, which were started after MAGIC was in progress, raised several doubts about such grafting. Skeletal myoblasts became skeletal myocytes in the heart rather than cardiomyocytes, and engrafted skeletal myocytes were not electromechanically coupled with native myocardium.27 The results of the MAGIC trial reinforced these issues: patients treated with autologous myoblast injections had cardiac arrhythmias and no significant improvement in ventricular contractile function.28

Early studies in animals suggested that bone marrow cells might have potential as cardiac regenerative therapeutics. Bone marrow cells injected into rodent hearts seemed at first to differentiate into cardiomyocytes in situ.29 Studies of human recipients of orthotopic heart transplants reported that cells with the recipient’s genotype were present in the transplanted heart.30,31 Many clinical trials followed these reports. Although injection with bone marrow cells was subsequently reported to be safe, the associated benefit was variable. In some trials, benefit was present, but short-lived.32 In other trials, no benefit was noted.22,23 A meta-analysis reported the aggregate long-term outcome from 29 randomised control trials studying the treatment of acute myocardial infarction with either an intracoronary injection of bone marrow cells or intravenous injection of granulocyte colony-stimulating factor (G-CSF) to mobilise bone marrow cells.24 This analysis showed a small improvement in left ventricular ejection fraction after injection of bone marrow cells (~3%).28 No significant benefit was associated with treatment with G-CSF.

A small improvement in left ventricular function after injection of bone marrow cells was also noted in patients enrolled in the REPAIR-AMI33 and STAR-heart34 studies, the largest randomised trials of injection of bone marrow cells in the context of acute and chronic myocardial infarction, respectively. The STAR-heart study reported a statistically significant decrease in mortality after injection of bone marrow cells compared with placebo. Notably, treatment with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers after myocardial infarction is associated with mortality benefit, even in the absence of a large improvement in left ventricular systolic function.20 This finding raises an important point about the criteria by which regenerative therapies are judged: full reversal of cardiomyocyte loss might not be necessary to change the natural course of heart failure. Large studies will be needed to further investigate the degree of mortality benefit, and cost effectiveness, of treatment with bone marrow cells.

The small and inconsistent benefit associated with treatment with bone marrow cells was initially attributed to inefficient delivery, because only 10% (or less) of delivered cells remained in the heart.29 Indeed, one meta-analysis suggested there might be a relation between bone marrow cell dose and therapeutic effect.34 Animal experiments started after the first wave of clinical trials proposed another issue: assumptions about plasticity of the bone marrow cells were incorrect.25 The reported change in cell morphology after injection into the heart, originally interpreted as transdifferentiation, was actually caused by fusion of injected and native cells.30 Haemopoietic stem cells were also noted to adopt a mature blood cell phenotype in the heart.31

More recent data suggest that the clinically observed benefit associated with injection of bone marrow cells is not the result of engraftment, but rather the release of paracrine factors.35 These findings raise questions about the plasticity of other non-cardiac cell types proposed as candidate regenerative therapeutics, including adipose-derived stem cells.36 Clinical trials involving such cells are in progress, including phase 1 trials of adipose-derived stem cells (NCT00426868, NCT00442806) and a phase 2 trial of mesenchymal cells (NCT00721045).

**Endogenous cardiac progenitor cells**

Augmentation of endogenous regenerative activity is a compelling strategy for cardiac repair. In theory, such amplification can be achieved with two distinct approaches. One approach would be to stimulate expansion of cardiomyocytes or putative cardiac progenitor cells with a drug or paracrine factor, in the same way that erythropoietin is given to stimulate bone marrow progenitor cells to produce erythrocytes. The second approach involves propagation of cardiac cells with regenerative potential ex vivo followed by implantation of these cells directly into an injured area.

Early attempts to identify stem cells in rodent hearts made use of several techniques and yielded various phenotypically distinct cell populations, generally termed cardiac progenitor cells.37,38 Several, but not all, of these populations express the c-Kit and Sca-1 proteins. Early studies suggested that transplantation of cells expressing c-Kit into the heart could stimulate formation of new blood vessels and myocardium.39 Suspension culture of cells obtained from human myocardial biopsy samples yields spheroid cell clusters (termed cardiospheres) that contain a mixed population of c-Kit-expressing and Sca-1-expressing cells. It was reported that cardiospheres could stimulate cardiac regeneration after infarction.40 These results led to the initiation of several phase 1 clinical trials involving heart-derived cells: ALCADIA (NCT00981006), SCIPIO (NCT00474461), and CADUCEUS (NCT00893360). Preliminary results from the SCIPIO and CADUCEUS trials were recently published.41-43 Both studies were designed to assess the feasibility and safety of intracoronary injection of autologous heart-derived cells after recent infarction. The SCIPIO study44 involved c-kit-expressing cells cultured from explanted atrial tissue, whereas the CADUCEUS study45 involved cardiospheres cultured from biopsy-obtained right ventricular tissue. Neither study showed a
significant increase in adverse events associated with cardiac cell injection, although more adverse events were noted in the treatment group of the CADUCEUS study. Both studies reported reduction in myocardial scar mass following cell treatment, but only the SCIPIO trial reported an improvement in left ventricular ejection fraction. The reported improvements should be viewed with caution, since the number of patients in the treatment arm of each study was small (16 in SCIPIO and 17 in CADUCEUS), and neither study included a placebo group because of the invasive nature of the treatment. Larger studies, powered to show clinically meaningful outcomes, will be needed to demonstrate the safety and efficacy of these treatment strategies.

Substantial gaps remain in our knowledge about cardiac progenitor cells and the mechanisms by which they might promote regeneration. Although c-Kit-positive cells are present at sites of cardiac injury, they do not have a known role in injury response. No consensus exists about phenotype definition or isolation technique of these populations, many of which have not been compared. Genetic fate mapping, a stringent scientific technique used to establish the derivatives of a progenitor cell population, suggests that c-Kit-positive cells and cardiospheres do not differentiate into cardiomyocytes. In mice, c-Kit-positive cells derived from the bone marrow augment cardiomyocyte renewal after infarction without direct transdifferentiation. This effect might be attributable to the actions of paracrine factors, released by the injected cells (ie, c-Kit-expressing cells or cardiospheres), on a distinct population of endogenous cardiac cells.

Genetic fate mapping and endogenous cardiac progenitor cells

The search for cardiac cells and paracrine factors that are capable of triggering cardiac repair has been challenging. Further progress will need more precise definitions of the phenotypes and biological roles of relevant cell populations. Use of genetic fate mapping to study embryonic cardiogenesis has eased the definition of progenitor populations and helped establish their roles in forming of discrete cardiac structures.

The heart contains a complex array of structures, including muscle, valves, arteries, veins, and a conduction system (figure 2). Distinct cell types make up each structure. Most cells in the adult heart are derived from mesoderm and a few are derived from cardiac neural crest. Early in embryogenesis, mesodermal cells that are destined to become part of the heart segregate into two anatomically distinct groups, termed the first and second heart fields. Cells in both heart fields express the Nkx2.5 protein. Genetic fate mapping studies in mice showed that progenitor cells in the second heart field, which is marked by expression of the Isl-1 protein, give rise to cardiomyocytes in the right and left atria, the right ventricle, the outflow tract, the proximal coronary arteries, and most of the conduction system. Cardiac neural crest progenitor cells, characterised by Pax3 expression, also contribute to growth of the outflow tract. Cells expressing Isl-1 can be differentiated into all cell lineages present in the heart. Knowledge of these cell populations helps investigators assess the molecular cues that establish cell fate decisions of progenitors in the second heart field. Renewal of Isl-1-positive cells is induced by ligands of the Wnt–β-catenin pathway, suggesting that fate decisions are made at the individual-cell scale. Multipotent cardiac progenitors expressing ISL-1 have also been produced from human embryonic stem cells in vitro. Left ventricular cardiomyocytes are derived from the first heart field, for which a unique phenotype marker has not been identified. Because most cases of heart failure are attributable to left ventricular failure, further characterisation of the first heart field is especially important.

Genetic fate mapping suggests that embryonic cardiogenesis proceeds according to a stem cell-based paradigm in which lineage-restricted progenitor cells give rise to the mosaic of cells present in the adult heart. After embryogenesis, mammalian cardiomyocytes seem to expand through symmetric division. An embryonic progenitor population that persists to adulthood might be involved in stimulation of cardiomyocyte division in the adult heart. This progenitor population, which is marked by expression of the Wt1 and Tbx18 proteins, resides primarily on the proepicardial surface during embryogenesis, and gives rise to fibroblasts, smooth muscle cells, and potentially cardiomyocytes, in all four heart chambers (figure 2). These Wt1-positive and Tbx18-positive cells persist in the epicardium of the adult heart and proliferate in response to myocardial injury, secreting trophic growth factors into the underlying myocardium. Activation of this cell population can also be primed with paracrine factors such as thymosin β4. This population might mediate the putative paracrine effects of cells studied in reported clinical trials, although this suggestion is unproven. Further investigation into the mechanisms by which this epicardial population could be used to effect myocardial repair is warranted.

The promise of induced pluripotent stem cells

Even if the phenotypes of cardiac cells with regenerative potential can be better defined, their rarity might make it difficult to generate enough cells to produce a clinically meaningful effect. Embryonic stem cells are an attractive source of starting material for cell-based therapies, mainly because they are self-renewing pluripotent cells that can be differentiated into tissues from all three germ layers. Cardiomyocytes produced from mouse embryonic stem cells in vitro are closer to the fetal phenotype than to the adult phenotype, but do show electromechanical coupling with native cardiomyocytes; however, orthotopic embryonic stem cells
Figure 2: Cardiac progenitor cell populations and their contributions to cardiac structures in the adult heart.

(A) Cardiac structures develop from embryonic progenitor cell populations in the first heart field (shaded green), the second heart field (shaded red), both heart fields (shaded purple), or epicardial progenitor lineage cells (shaded blue). Neural crest cells also contribute to the great vessels and outflow tract. Isl-1 expression marks the multipotent progenitors of cardiomyocytes in the second heart field, and other protein expression profiles mark downstream progenitors and differentiated tissue types.

(B) Induced pluripotent stem cell technology. In-vitro reprogramming of terminally differentiated fibroblasts with KLF4, OCT4, SOX2, and C-MYC leads to a pluripotent phenotype analogous to an embryonic stem cell, potentially allowing future differentiation. Although reliable, such reprogramming is labour-intensive and slow. An alternative technique (shown beneath the dashed line) uses Klf4, Oct4, and Sox2 to reprogramme terminally differentiated embryonic fibroblasts into a usable intermediary (but non-pluripotent) cell that can be differentiated into cardiomyocyte phenotype in less than half the time of pluripotent stem cell technique.
are probably not suitable for clinical application, because of ethical worries, potential genetic instability, and requisite immunosuppression therapy. Takahashi and Yamanaka recently designed a technique for in-vitro reprogramming of terminally differentiated cells, such as skin fibroblasts, into pluripotent cells that closely resemble embryonic stem cells. These reprogrammed cells, termed induced pluripotent stem cells, can be derived from individual patients. Cardiac cells can then be produced from induced pluripotent stem cells in vitro. This technique makes possible autologous cell transplantation, with a theoretical reduction in risk of immune rejection.41

Several issues with induced pluripotent stem cell technology need to be resolved before clinical use is possible. The originally reported method for production of such cells used integration of viruses to express four factors that drive reprogramming (figure 2). Because of the risk of malignant transformation associated with integrating viruses, an alternative method is needed,42 for which several candidates have been reported.43,44 Another issue is that of acquisition of the source cell. Induced pluripotent stem cells retain epigenetic memory of the cell type from which they were derived. Thus, cardiomyocytes can more readily be produced from induced pluripotent stem cells derived from the ventricular myocyte than from those derived from fibroblast tissue.45 This feature emphasises the subtle, but important, differences between induced pluripotent stem cells and embryonic stem cells. Before provision of induced pluripotent stem cell-derived cardiac cells to a patient, institution of a reliable method for removal of any pluripotent cells is needed.46 Furthermore, immunosuppressive therapy might also be necessary, because induced pluripotent stem cells can elicit an immune response when transplanted between genetically identical mice.47

Difficulties encountered with induced pluripotent stem cells inspired the development of methods for turning fibroblasts directly into cardiomyocytes without regression to a pluripotent state. The operational assumption in this approach is that travelling a shorter developmental distance might help avoid the issues associated with pluripotent cells.48-50 Direct transdifferentiation of mouse embryonic fibroblasts into cardiomyocytes without transit through a pluripotent intermediate has been reported (figure 2).49 This strategy has issues of its own; for example, cardiomyocytes produced in this way express only the atrial isoform of myosin.

**Paracrine factors in cardiac regeneration**

Paracrine factors, notably those in the renin–angiotensin system, have key roles in cardiac pathophysiological mechanisms.51 The benefit associated with bone marrow cell therapy might be attributable to paracrine factors, but neither the identity nor the actions of these putative factors are known. Several possible activities have been proposed, including activation of putative endogenous cardiac progenitor cells, direct stimulation of cardiomyocyte division, and modification of the tissue niche (ie, increase neovascularisation and reduce scar burden).52 Use of paracrine factors to mobilise cardiac cells with regenerative potential is a compelling treatment strategy, especially because it is associated with a potentially smaller risk of tissue disruption than is cell injection.

Growth factors investigated as candidate cardiac therapies fall into several functional categories, such as those that promote angiogenesis (eg, vascular endothelial growth factor, fibroblast growth factor, and stromal cell derived factor 1), inhibit apoptosis (eg, hepatocyte growth factor and platelet-derived growth factor), mobilise progenitor cells (eg, G-CSF), and stimulate myocyte proliferation (eg, peristin and neuregulin). The potential benefit of growth factor infusion, either in combination with cell infusion or as standalone therapy, is under investigation, and various preclinical studies of growth factor infusion after myocardial infarction have been undertaken.71 Paracrine factors that inhibit inflammation might reduce scar formation and thus improve the suitability of the niche for cardiomyocyte growth; however, reduction in fibrosis could destabilise myocardium after infarction and increase the risk of rupture.

**Delivery of regenerative therapy**

A safe, effective, and practical delivery system is crucial to the success of cardiac regenerative therapy. Such a platform would have to ensure reliable delivery of a sufficient amount of the therapeutics to trigger regeneration, have good visualisation of the target area, and provide specific delivery to the target area with minimal off-target delivery and little or no risk of haematogenous dissemination.72 Several delivery techniques have been reported, ranging from direct intramyocardial injection in the context of open-chest surgery to various intravascular, catheter-based methods (figure 3).

Perhaps the most feared long-term outcome of the haematogenous dissemination of progenitor cells is metastatic tumour formation. Intramyocardial injection from the epicardial approach is probably associated with a lower risk of haematogenous spread than is injection from the intravascular approach. Most areas of the heart are accessible from the epicardial surface, apart from the interventricular and interatrial septa. Administration of regenerative therapies via the epicardial approach was first undertaken during open-chest surgery. The procedural morbidity of the open-chest approach can offset the decrease in risk of haematogenous dissemination. Use of this approach is therefore difficult to justify unless another cardiac surgical procedure, such as coronary artery bypass grafting, is indicated.73 Morbidity associated with the open-chest approach motivated investigators to assess less invasive, catheter-based techniques. A percutaneous transarterial approach
has been used for both intramyocardial and intracoronary injections. Nonetheless, a decrease in procedural morbidity is offset by an increase in the risk of haematogenous dissemination and an inability to directly visualise the affected area. Intravascular delivery of therapeutics is inefficient, because the injected cells or growth factors need to move through the vessel wall. Moreover, the targeted area of myocardium is not accessible via the transarterial approach if the artery serving that region is occluded.

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An ideal delivery system would combine advantages of open-chest approach (specific delivery) and percutaneous approach (low procedural morbidity).

Development of videoscopic catheter technology, which allows the operator to view internal anatomy, has eased the development of minimally invasive strategies for delivery of regenerative therapy to the epicardial surface of the heart. An early version of this technology, involving insertion of catheters into the pericardial space through small incisions in the subxiphoid region, has been reported. This percutaneous catheter-based system provides the advantages of the epicardial approach (ie, direct visualisation and a reduced risk of haematogenous dissemination compared with intravascular injection) without the morbidity of open-chest surgery.

All reported cardiac-injection techniques are associated with low retention of delivered cells. Retention is higher with intramyocardial injection than with intravascular
injection, but is poor nonetheless. Indeed, a cell retention in pigs with intramyocardial injection of 11% has been reported, which was better than the 2-6% reported with coronary arterial injection or 3-2% with coronary sinus injection. Hydrogels designed to solidify at the time of injection can improve cell retention and engraftment. Studies in animals suggest that injection of hydrogel formulations alone can improve repair after infarction. Catheter compatibility is a key issue with biopolymer-based hydrogels, which solidify rapidly and therefore tend to do so within the delivery catheter. This issue will need to be addressed before clinical application is practical.

Cardiac tissue engineering
Successful reconstitution of damaged cardiac tissue cannot rely solely on regeneration of cardiomyocytes because the architecture of myocardium is complex. Cardiomyocytes in the adult heart are oriented end-to-end in fibres, which are woven into anisotropically oriented sheets whose organisation forms the basis for chamber contraction. Among these sheets are complex webs of fibroblasts, blood vessels, and conduction-system tissue. Spatial organisation of these cells during embryogenesis is thought to involve signals between cells and from the extracellular matrix that make up the tissue niche. In the adult, this niche is thought to be crucial to cardiac repair. Consistent with this notion are the reports that embryonic stem cell-derived cardiomyocytes engraft more efficiently into native myocardium than into scar tissue.

Perhaps the most striking example of the importance of extracellular matrix to survival of injected cells was a study in which a whole rat heart was decellularised and then repopulated with neonatal cardiomyocytes, resulting in a beating heart. Application of biopolymer patches, impregnated with growth factors and progenitor cells, to the epicardial surface of myocardial scar stimulates neovascularisation and decreases scar size. Bioactive polymer technology makes possible the creation of patches that selectively release growth factors in response to matrix metalloproteinases expressed at the site of myocardial infarction.

One of the main drawbacks of biopolymer-based technology is the potential for adverse host reaction to biomaterials. This issue led to the development of scaffold-free tissue-engineering technology. These so-called second-generation patches are made up only of cells and the extracellular matrix that these cells secrete. One new patch contains only human cardiomyocytes, endothelial cells, and stromal cells. In a rat model, vessels in such patches form effective anastomoses with native vessels, and patch cardiomyocytes are electromechanically associated with adjoining myocardium. Cardiac tissue-engineering technology is a crucial complement to the study of cardiac progenitor populations. Progress in cardiac tissue engineering in the past few years has been very rapid, but these techniques are still at an early stage. Preparation of engineered tissue remains a very labour-intensive process that will need to be streamlined before large-scale clinical application is practical.

Perspectives
The heart is made up of a complex mosaic of distinct anatomical elements that are substantially disrupted in cardiac injury. Because of this complexity, restoration of cardiac function would need recreation of the native architecture of the heart, not just regeneration of one cell type. An ideal cardiac regenerative therapy would possess a key cell and paracrine factor combination, a cardiac tissue niche optimised to enhance cell engraftment and differentiation, and a safe, minimally invasive delivery procedure that introduces the regenerative therapy specifically to the affected area with the least risk of acute and long-term side effects. Because of these distinct challenges, the design elements of a clinically meaningful regenerative therapy strategy for advanced heart failure will probably reside at the intersection of the discrete arenas of stem cell biology, tissue engineering, transplantation, grafting, rejection biology, clinical cardiovascular medicine, and device technology. Furthermore, creation of interdisciplinary teams, including partnerships between academia and the private sector, will probably be needed. Physician–scientists who have world-class clinical training in cardiovascular medicine, together with state-of-the-art knowledge of stem cell and regenerative biology, will be key members of these teams. Regenerative therapy in cardiac disease is in an important phase: breaking down of traditional barriers between individual areas of specialisation will be challenging, but necessary, if we are to move beyond stem cell biology towards the development of true cardiovascular regenerative therapy. To foster this sort of multidisciplinary collaboration, a parallel regenerative effort to change the existing academic culture and environment might be necessary. The fate of the next generation of leading physician–scientists in the specialty, and the fate of patients with heart failure, will probably hinge on the outcome.

Contributors
LMP and KRC did the literature search, wrote the report, and drew the figures. MM and JNR provided guidance about the content of the text and figures.

Conflicts of interest
We declare that we have no conflicts of interest.

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