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# Regenerating the field of cardiovascular cell therapy

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**The retraction of >30 falsified studies by Anversa et al. has had a disheartening impact on the cardiac cell therapeutics field. The premise of heart muscle regeneration by the transdifferentiation of bone marrow cells or putative adult resident cardiac progenitors has been largely disproven. Over the past 18 years, a generation of physicians and scientists has lost years chasing these studies, and patients have been placed at risk with little scientific grounding. Funding agencies invested hundreds of millions of dollars in irreproducible work, and both academic institutions and the scientific community ignored troubling signals over a decade of questionable work. Our collective retrospective analysis identifies preventable problems at the level of the editorial and peer-review process, funding agencies and academic institutions. This Perspective provides a chronology of the forces that led to this scientific debacle, integrating direct knowledge of the process. We suggest a science-driven path forward that includes multiple novel approaches to the problem of heart muscle regeneration.**

The ultimate goal of regenerative medicine is to repair organs and tissues after life-threatening injury or disease. But for regenerative medicine directed to the heart, the field of cardiac cell therapy itself cries out for 'regeneration'—damaged as it is by the retraction of over 30 papers touting the promise of putative adult blood-forming or heart endogenous stem cells for healing myocardial injury[1]. Given the crushing medical complications of patients with end-stage heart failure, the marked increase in prevalence, and the severe limitation of donor hearts for transplantation, the retracted studies, published by Anversa and colleagues from 2001 onward, created excitement among patients, physicians and scientists. At the time, outside of blood stem cell therapy, cardiac regeneration may have been the most compelling case for translating fundamental advances in stem cell science into clinical practice. Consequently, after dozens of clinical studies and/or trials and almost two decades of largely fruitless work, the degradation of public confidence in this field is palpable, along with the dashed hopes of millions of patients worldwide with end-stage heart disease. At this pivotal moment for the field of regenerative cardiovascular medicine, it is timely to bring a clear and frank retrospective analysis of the ontogeny of this biomedical tragedy. Our sole aim here is to help avoid a repeat, in any area of regenerative medicine, and to chart a corrective course for cardiac cell therapy.

## A flawed idea takes hold

How did the proposition that blood-forming stem cells could transdifferentiate to most or all nonblood tissues take hold? This field of dreams has its roots in the late 1990s—a remarkably productive time in stem cell biology. Dolly the sheep was cloned by somatic cell nuclear transfer by Wilmut and colleagues[2], proving that an adult cell could be reprogrammed to totipotency[3,4], and Thomson's group[5] isolated the first lines of human embryonic stem cells (hESCs)[5]. Less discussed nowadays is another prevalent hypothesis of the era: the notion that adult stem cells are highly plastic and, when transplanted into new environments, can 'transdifferentiate' into cells that belong to their new host site. In the early 2000s, several reports posited the transdifferentiation of bone marrow hematopoietic stem cells into brain, lung and liver, for example[6–10]. Although these studies were subsequently disproven (brain and lung) or shown to result from cell fusion (brain and liver)[11,12], they seeded the 'anything is possible' zeitgeist that emerged.

Delving a bit deeper, it was clear at the time that these iconoclastic studies ran counter to several decades of work in hematopoiesis and transplantation biology. Blood or hematopoietic stem cells (HSCs) had been prospectively isolated and shown to self-renew HSCs as well as to give rise to all blood cell types for life. In the 1990s, HSC transplants to replace bone marrow, mobilized peripheral blood or umbilical cord blood transplants were tested in experimental systems and in autologous transplant clinical trials (for a review, see ref. [13]). In 2005, a study tracing HSCs transplanted into mice found no evidence that HSCs can transdifferentiate into tissues other than blood cell types[14]. Even in parabiotic mice, with linked circulations and mixing of blood cells, a 2002 study did not observe appreciable transdifferentiation of circulating HSCs into nonhematopoietic tissues[15]. Nevertheless, several papers were subsequently published that countered those data and claimed that HSCs can normally transdifferentiate to many distinct cell types.

The publication of these reports represents a failure of many entities to take the advice of stem cell biologists who were experts in the field. These entities include the investigators who claimed that HSCs are plastic and can assume other tissue fates after injection into animals[16]. They include high-impact journals that accepted

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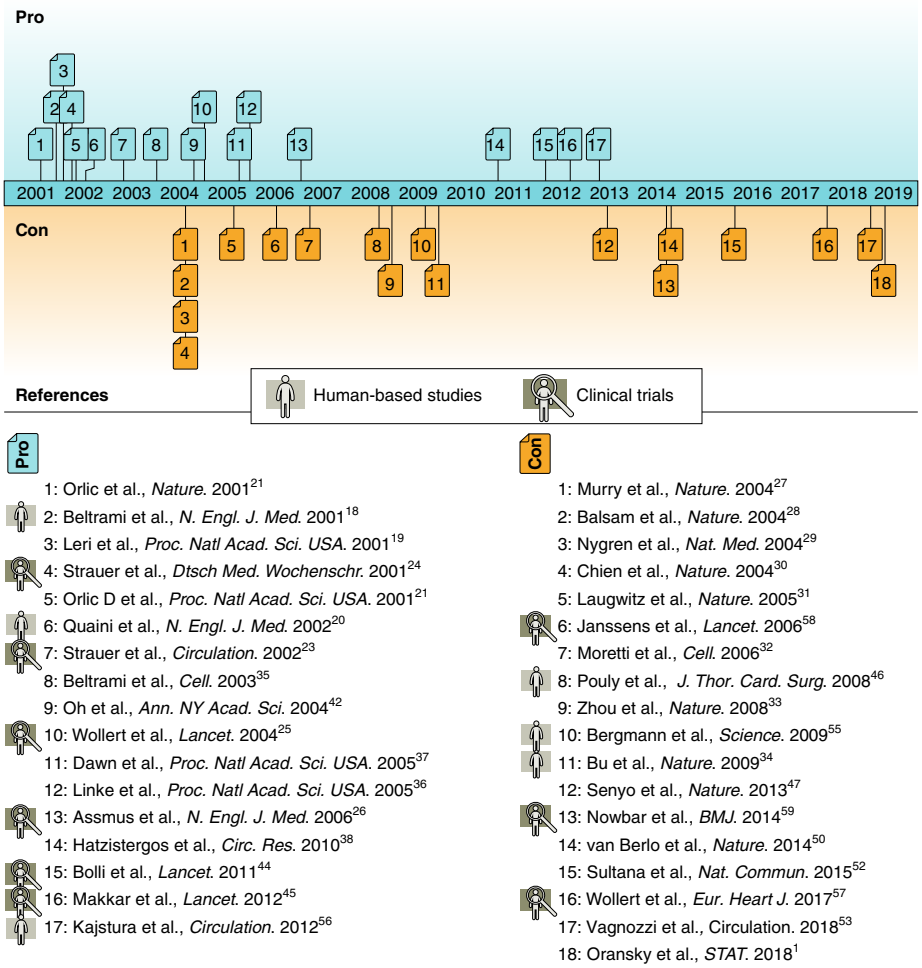


**Fig. 1 | Timeline of fundamental findings and preclinical outcomes supporting or contradicting the notion of cardiac stem/progenitor cell therapy.** Human studies and clinical trials are indicated with icons.

papers claiming transdifferentiation of HSCs or blood cells to heart or brain or intestines or skeletal muscle, despite multiple negative reviews by stem cell experts. Some of these journals simultaneously rejected papers showing the first prospective isolation of human fetal brain stem cells, which were restricted to regenerating the tissue from which they were obtained, while publishing a claim that brain stem cells make blood[17]. Culpable entities also include funding agencies that awarded large grants to basic and clinical scientists who proposed that regeneration in humans can be achieved by remarkably simple strategies such as putting bone marrow cells into injured tissues and seeing what sticks.

## A short and unfortunate history

Pathologists had long taught that the heart is among the least regenerative organs in the human body, a view based on centuries of clinical and experimental observations. In the late nineties, Anversa's group began to challenge these teachings, positing instead that the heart is a self-renewing organ. Beginning in 2001, they published a series of papers claiming that human hearts contain high levels of mitotic cardiomyocytes[18,19], that circulating cells become cardiomyocytes (using female-to-male heart transplant patients)[20] and that bone marrow cells induced by blood-forming cytokines enhance heart regeneration in mice[21]. In 2001, a controversial paper from the group claimed that the injured heart can be robustly regenerated by transplantation of bone marrow stem cells expressing the surface marker c-kit[22] (Fig. 1). Although this conclusion was based on only a single mouse study[22], the approach was quickly translated into a clinical trial that was completed and published the following year[23] (Fig. 1). The simplicity and purported robustness of the initial mouse work, coupled with few clinical regulatory barriers for autologous transplantation beyond a local institutional review board, along with the documented efficacy of acute intervention (angioplasty) during acute myocardial infarction, quickly led to anecdotal and large-scale clinical studies worldwide[24–26], several of which were published in major medical journals.

Meanwhile, a number of investigators had difficulty reproducing several of the Anversa group's original experimental findings. Three papers published in 2004 provided direct, unequivocal evidence that the transplanted bone marrow did not transdifferentiate into cardiac muscle[27–29] as initially reported (Fig. 1). While the basis for this discrepancy was not clear at the time, it was noted that the initial findings were based entirely on immunostaining to show the intersection of the bone marrow lineage marker (green fluorescent protein) with the cardiomyocyte marker (myosin)[30]. Immunostaining is a more subjective and less rigorous test than genetic recombination approaches, and the difficulty is compounded by the high nonspecific background staining in cardiac muscle tissue. To obviate this barrier, in vivo lineage tracing with Cre-*lox* technology was being established to define heart progenitors that build both muscle and nonmuscle components in the heart[31–34] (Fig. 1), a method that was to become de rigeur in establishing the identity of putative heart stem/progenitor cells. Notably, the embryonic cardiomyogenic progenitor cells that build most of the mammalian heart muscle were found to be completely absent in the adult heart[31].

While these findings all contradicted Anversa's claims and escalated the scientific controversy, they did little to slow the trajectory of the clinical studies, and a handful of uncontrolled clinical trials reported a small beneficial signal (Fig. 1). These results were subsequently attributed to unknown 'paracrine cues' rather than to direct cellular contributions to new heart muscle. As discussed below, it was argued that paracrine effects might improve cardiac function by enhancing vascularization, reducing fibrosis and promoting host cardiomyocyte survival after myocardial infarction.

## Pivoting to cardiac stem cells

Although the clinical community continued with the study of bone marrow cells, the Anversa group pivoted sharply from the hypothesis of bone marrow transdifferentiation to instead proposing that the heart itself contains a robust stem cell population expressing the same c-kit marker they had used in the bone marrow[35]. Once again, the claim was based on immunostaining and transplantation, without any demonstration of the resident adult cardiac c-kit$^+$ cell's innate 'stemness' by lineage tracing via Cre recombinase (Fig. 1). They argued that these adult 'cardiac stem cells', or CSCs, are inexhaustibly expandable and responsible for the normal, large-scale turnover of cardiac muscle cells in rodents and humans[35]. Moreover, they asserted that CSCs can be easily identified and isolated for transplantation[35–37]. These bold and potentially important reports led to a flurry of studies in laboratories collaborating with Anversa, which reported positive, though less dramatic, heart regeneration with cardiac c-kit$^+$ cells[38–41]. Simultaneously, other groups isolated still more putative CSCs. Some used additional markers from mouse hematopoiesis, such as Sca-1[42], whereas others used the ability to grow putative CSCs in three-dimensional clusters called cardiospheres[43].

On the basis of this evidence, clinical trials of CSCs were undertaken by several groups. For example, working with Anversa's group, the Bolli group delivered cardiac c-kit$^+$ cells to the hearts of patients with chronic ischemic heart failure[44]. They reported good safety profiles and substantial improvements in cardiac function in the treated group versus virtually none in controls. As discussed below, the veracity of these findings are now questioned, and the study has been earmarked with a cautionary warning related to data analysis. Marbán's group[45] tested a second putative cardiac stem cell, the cardiosphere-derived cell, in patients with recent myocardial infarction. They reported that these cells are safe but do not provide benefits to global cardiac function. Both of these populations are still under clinical investigation by their respective groups.

Most recently, the US National Institutes of Health (NIH) sponsored a clinical trial, named CONCERT-HF (NCT02501811), comparing cardiac c-kit$^+$ cells, bone marrow mesenchymal stromal cells, and a combination of the two for patients with chronic ischemic heart failure. As of this writing, patient enrollment was largely complete, and one patient has died of bleeding complications from the cardiac biopsy needed for isolation of the c-kit$^+$ cells. We return to the CONCERT-HF trial below.

## The adult resident cardiac stem cell hypothesis unravels

Over the next several years, multiple groups had difficulty corroborating the stemness of the cardiac c-kit$^+$ cell. Menasché et al.[46] reported that, in patients with heart failure, cardiac c-kit$^+$ cells are actually mast cells (i.e., mature, resident tissue leukocytes). Lee's group[47] used a highly sensitive technique, multi-isotope mass spectroscopy, to demonstrate that adult mouse hearts turn over very slowly, ~1% per year, and that this turnover can be accounted for entirely by division of pre-existing cardiomyocytes. This corroborated earlier findings from Field's group[48] in the nineties and Rumyantzev's group[49] in the sixties using [$^3$H]thymidine to measure DNA synthesis rates in cardiomyocytes.

In 2014, van Berlo et al.[50] published a much-awaited study on genetically based lineage tracing of the cardiac c-kit$^+$ cell using Cre recombinase in mice. In these mice, administration of the drug tamoxifen induces a heritable genetic tag in cells expressing c-kit. By performing a drug-free 'chase' period, the fate of the tagged cells can be followed in health and disease. The authors showed that, during normal aging or following injury such as myocardial infarction, the level of new cardiomyocyte formation from c-kit$^+$ cells is fewer than 1 in 10,000. In other words, there was no basis for the claim that the c-kit$^+$ cell is a cardiac muscle progenitor. Although the veracity of this study was disputed by Anversa and collaborators[51], other groups provided further independent evidence against the c-kit$^+$ cardiac stem cell hypothesis[52], including the demonstration that Sca-1$^+$ cells also are not cardiac progenitors[53,54].

A paper by Bergmann et al.[55] estimated the turnover of human cardiomyocytes by studying the incorporation of radioactive carbon, derived from atmospheric testing of nuclear weapons, into cardiomyocyte DNA. They found that cardiomyocytes are, on average, a little younger than the individual's age, estimating annual turnover rates of 1% at age 20 and 0.5% at age 50. This directly challenged the notion that the human heart is a rapidly self-renewing organ. Anversa's group quickly disputed this report and repeated the study. Their findings[56] directly contradicted those of Bergmann et al.[55] in showing equally rapid turnover of cardiomyocytes, vascular cells and connective tissue cells (6–8 times in a 60-year period). To most in the field, it seemed to be a confounding stalemate; instead, a dramatic break lay ahead.

Radiocarbon dating requires sophisticated mass spectrometry, which was performed for both studies[55,56] by scientists at Lawrence Livermore National Laboratory (LLNL). When the Anversa group's paper[56] was published in *Circulation*, the LLNL scientists were made aware of discrepancies between the original spectra they had generated and those that the Anversa group had reported in their paper. The discrepancies favored the Anversa group's hypothesis of rapid turnover of human cardiomyocytes. Unable to reconcile the published data with good scientific practice, the LLNL reported their concern to the NIH Office of Research Integrity.

Subsequently, the Brigham and Women's Hospital launched a 5-year investigation that ultimately concluded that Piero Anversa, Jan Kajstura and Annarosa Leri had committed widespread scientific misconduct. As of this writing, the Brigham and Women's Hospital has announced that 31 papers will be retracted[1]. A complete list has not been released, but *Circulation Research* and *Circulation* have recently retracted 13 papers. In addition, the NIH decided to halt enrollment in the CONCERT-HF clinical trial. The agency and the data safety monitoring board review are now reviewing the scientific basis for the trial (Fig. 1).

## Pivoting to the paracrine hypothesis

Although the animal studies published in 2004 (refs. [27–29]) clearly showed that bone marrow cells do not convert to cardiomyocytes, clinical studies with bone marrow transplantation into the heart continued unabated (Fig. 1). Indeed, a new series of clinical studies with a diverse set of autologous and allogeneic cells, including bone marrow mesenchymal stromal cells, adipose stromal cells and, as discussed above, resident cardiac cells, was launched. However, the scientific rationale for these studies had shifted entirely, from bona fide stem cells that transdifferentiate into cardiomyocytes to the suggestion that the cells live for only a few days in their new site, secreting unknown, beneficial paracrine factors to the surrounding injured heart tissue before their death. This paracrine hypothesis arose to reconcile the findings that CSCs largely did not engraft in the heart, but did, in animal studies, have some beneficial effects on cardiac function. Aside from demonstrations that paracrine effects can be reproduced by extracellular vesicles from cultured cells, there has been little investigation into the mechanisms.

**Table 1 | Five-dimensional framework guidance for a cardiac stem cell therapy**

| Assessment of five R's | Stem cell therapy for cardiac regeneration |
|---|---|
| **Right target** | • The selected cell is a cardiac progenitor cell, known to be present in the embryonic and/or postnatal heart, where it differentiates into ventricular cardiomyocytes<br>• The selected cell can be identified using distinct molecular markers to establish a clear fingerprint of the progenitor cell and can be purified to necessary homogeneity with batch-to-batch consistency<br>• The selected cell can differentiate in vivo into a mature ventricular cardiomyocyte with structural and functional similarities to an adult mature cardiomyocyte<br>• Efficacy in terms of improved cardiac function can be established in a rodent and large animal model before progressing into clinical trials |
| **Right tissue** | • After direct intramyocardial injection, the selected cells are viable, are fully integrated into the myocardium, and differentiate to mature cardiomyocytes. There is minimal leakage of the selected cells into the circulation and no distribution to other tissues |
| **Right safety** | • CMC, QC and analytical methods of the drug product fulfill regulatory requirements and are stable and consistent<br>• A pure, homogeneous, well-defined, genetically stable cell population that integrates and couples electrically to the host myocardium is produced to secure long-term grafting and avoid arrhythmias and formation of teratomas<br>• Ideally, the selected cells are engineered to be 'universal' and equipped with a 'kill switch' to avoid attack by the immune system and uncontrolled cell proliferation, respectively. |
| **Right patient** | • Randomized, placebo-controlled, proof-of-principle clinical trial is performed in patients with poor cardiac contraction and a high unmet medical need; i.e., patients undergoing CABG, patients on mechanical pumps or patients with severe heart failure<br>• The same cGMP cell line is used for clinical trials and the GLP toxicology studies. All work is performed under GMP conditions with all CMC aspects, including scalability, consistency and QC of the drug product, needed to fulfill regulatory requirements globally<br>• Target engagement and proof of mechanism can be established with imaging methods |
| **Right translation** | • Large-scale manufacturing to meet demand in large global clinical trials and for a commercial product in line with regulatory requirements<br>• Supply chain for materials and cells, preferably cryopreserved cells; consistency between batches and quality attributes of cells clearly established<br>• Clear fit with global healthcare systems: for example, logistics and processes that fit with commercial needs<br>• Drug product administration procedure with a suitable delivery device established at hospitals<br>• Clear reimbursement models |

Framework based on Morgan et al.[60]. CMC, chemistry manufacturing and controls; QC, quality control; CABG, coronary artery bypass graft; GLP, good laboratory practice; GMP, good manufacturing practice.

While a detailed analysis of the clinical trials of adult cells is beyond the scope of this Perspective, a few lessons emerge. Bone marrow trials have been consistently marginal, ambiguous or negative in showing an impact on cardiac function or patient outcomes, such as mortality or rehospitalization. Two independent double-blind, placebo-controlled studies have failed to show improvement in cardiac function[57,58] (Fig. 1). Moreover, the studies with the most technical errors have reported the most positive functional effects, suggesting that a lack of rigor may have contributed to a positive bias[59]. While there are only a few reports of trials of cardiac-derived cells, the single study that claimed a positive benefit was from the Anversa and Bolli groups[44] and has been flagged with an 'expression of concern' by *The Lancet* because of data irregularities. In several cases, the only way to rationalize continuing the studies was via retrospective analyses (for example, finding subgroups that benefitted from high cell doses), although the studies were never powered to define the effects of dose escalation, raising the issue of 'cherry picking' from datasets.

## Surveying the damage and facilitating factors

In hindsight, a careful examination of the circumstances that enabled and perpetuated this scientific and clinical tragedy is warranted. The cost has been enormous: it has wasted research and clinical trial support (likely >$250 million) that could have been used to support more promising technological approaches for heart repair; it has had a demoralizing effect on a generation of young cardiovascular physicians and scientists who chased the Anversa studies; it has shaken public confidence in regenerative cardiology; and, most importantly, it has put in harm's way thousands of patients who underwent a risky clinical procedure under the false pretense that adult cell–based cardiac therapy was supported by robust and compelling preclinical evidence.

Accordingly, it would seem incumbent upon all the institutions involved to carefully consider how to prevent similar missteps in the future by implementing more professional and rigorous procedures (Table 1)[60]. A key facilitating factor clearly relates to the staggering, unmet clinical need for patients with end-stage heart disease, coupled with the alignment of the hopes of patients, physicians and scientists. The hopes of desperate patients are understandable, but as physicians and scientists, we need to temper our exuberance with professional skepticism and to demand rigorous evidence before initiating clinical trials. Institutions should adhere to the practice of obtaining letters and opinions from unbiased outside experts when hiring faculty. Proponents of adult cells for cardiac repair could have integrated state-of-the-art stem cell technology, such as lineage tracing, to clarify the role of c-kit$^+$ cells at an early stage. The paracrine hypothesis could have been thoroughly investigated mechanistically in preclinical models before considering clinical translation of the approach.

In many ways, the protracted time frame for correcting errors in the scientific literature also points to major deficiencies in the peer review systems that control publication, funding and academic appointments. Scientific publication has a strong bias against negative data, and there is an increasing predilection to publish studies that tout breakthroughs or iconoclastic results. In many cases, the retracted Anversa studies were reviewed and/or handled by editorial groups that included active collaborators and coauthors, a potential conflict of interest. In other cases, the turnaround times were remarkably short (for example, one day from submission to acceptance), raising questions of rigorous and independent peer review. In retrospect, given that the core stem cell technology is shared across fields and diseases, the publication review system, as well as the faculty appointment system, would have benefited from stronger independent input from researchers outside the cardiovascular biomedical community.

Science policy may have also played a role in this affair. Severe restrictions on federal funding for hESC work were in place from 2001–2008, and the American Heart Association adopted a policy not to fund hESC work, a policy that continues to this day. These policies created a substantial funding barrier against the entire area of pluripotent stem cell research, and they emphasized the view that adult cells were suitable as regenerative therapeutic agents.

## The road ahead

One of the clear lessons learned from this saga is the need for a full recognition of the multiple challenges and complexities of cardiac cell therapy. The successful development of cardiac cell–based therapeutics into standard clinical practice will require that multiple scientific, clinical and financial hurdles be addressed, including identification of the optimal cell type, tractable in vivo delivery systems, long-term functional tissue formation with safety parameters, proof of concept in large-animal models that more closely mimic the clinical in vivo context, conversion to scalable clinical manufacturing standards, quality control related to batch-to-batch variation of a complex biological agent, management of potential side effects, identification of the ideal patient subsets, and the development of a viable business model for cell therapies (Table 1)[60]. But first, there must be a valid discovery that can be independently replicated.

In this regard, the only demonstrated and robust source of either authentic human cardiomyogenic progenitors or differentiated cardiomyocytes is human pluripotent stem cells. Multiple independent laboratories have developed protocols that can generate virtually any cardiomyocyte progenitor or subtype (ventricular, atrial, pacemaker)[61–65], and now there is evidence of clear functionality in vivo in large animal models, including primates[66], as well as initial clinical safety data[67]. While it is still in early days, with serious issues remaining (for example, arrhythmogenesis, immunosuppression, optimal delivery, cell purification, scalability, batch-to-batch variation, long-term viability; Table 1), each of these issues can be rigorously attacked experimentally. For some academic investigators, this means forging partnerships with the biopharmaceutical industry, which has sophisticated expertise in regulatory affairs, safety and toxicology, and rigorous clinical trial design. Such real-time, interactive collaborations may prove to be preferable to solo academic efforts in developing an entirely novel, highly complex class of therapeutic.

Early experimental studies from multiple independent laboratories have now established a new generation of strategies to trigger heart repair and regeneration, including the use of human heart progenitors and cardiomyocytes derived from pluripotent stem cells; the tissue engineering of heart patches; paracrine-factor therapeutics with proteins, genes and modified mRNA (for a review, see ref. [68]); direct reprogramming of fibroblasts to cardiac muscle[69]; and the release of cell-cycle checkpoints to promote cardiomyocyte replication[70]. Each of these approaches has inherent strengths and weaknesses, which again can be critically addressed through rigorous experimentation. A detailed and reproducible data package could prompt the private sector to catalytically move a project forward in such a way that rigorous regulatory, safety, quality control of complex cell based therapeutic, and scalability will be insured before initiating first-in-human clinical studies.

It is always difficult to balance the promise and optimism of cardiac cell therapy with its scientific, regulatory and financial challenges. The time frame for routine adoption into clinical practice could be anywhere from 5 to 25 years. However, one thing is clear. Just as in the case of gene therapy and cancer immunotherapy, it is likely to require the intersection of cross-disciplinary discoveries, long-term funding, and academic–private sector partnerships. With a stronger commitment to a science-driven approach to the challenges of heart regenerative therapeutics, perhaps we can provide renewed hope for patients with advanced heart failure.

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## Competing interests

K.R.C. is a scientific founder and equity holder in Moderna Therapeutics and Procella Therapeutics, and chair of the External Science Panel for AstraZeneca. R.F.-D. is an employee of AstraZeneca. J.F. is an advisor to 10XGenomics. D.A.M. is cofounder of Semma Therapeutics. C.E.M. is a scientific founder and equity holder in Cytocardia. I.L.W. is a cofounder of, director of, stockholder in and consultant to Forty Seven Inc, a company currently devoted to cancer immunotherapies with antibodies to macrophage checkpoint inhibitors.

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