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A mechanistic roadmap for the clinical application of cardiac cell therapies

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Abstract

The development of cells for regenerative therapy has encountered many pitfalls on its path to clinical translation. In cardiology, clinical studies of heart-targeted cell therapies began two decades ago, yet progress towards reaching an approved product has been slow. In this Perspective, I provide an overview of recent cardiac cell therapies, with a focus on the hurdles limiting the translation of cell products from research laboratories to clinical practice. By focusing on heart failure as a target indication, I argue that strategies for overcoming limitations in clinical translation require an increasing emphasis on mechanism-supported efficacy, rather than on phenomenological observations. As research progresses from cells to paracrine mechanisms to defined factors, identifying those defined factors that are involved in achieving superior therapeutic efficacy will better inform the use of cells as therapeutic candidates. The next generation of cell-free biologics may provide the benefits of cell therapy without the intrinsic limitations of whole-cell products.

Despite major advances in pharmacology and device therapy, heart disease — specifically heart failure, the deadliest form — remains an increasing major public health challenge¹. The dominant form of injury to the human heart is ischaemic: throm-bosis of a coronary artery leads to heart-tissue necrosis — a process commonly known as myocardial infarction. In adult mammals, the default response to myocardial infarction is scar formation, but neonatal mammals can regenerate the myocardium for a few days after birth. One goal of regenerative cardiology, which could in principle be achieved through cell therapies, is to take advantage of this developmental programme to convert the fibrotic response to a regenerative one in patients with myocardial infarction² (Fig. 1). The canonical approach to this objective posits that transplanted stem cells or progenitor cells will engraft, proliferate and differentiate into new healthy tissue. Conversely, transplanted cells may also activate beneficial, non-canonical mechanisms, including triggering anti-fibrotic and anti-inflammatory processes that potentiate the overall healing response. Therefore, cell therapy has the potential to be a game changer in the treatment of heart failure, as none of the treatments approved for this indication to date reverse the pathology at a fundamental level³. The possibility of regenerating sufficient healthy myocardium to enable stabilization, or

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even regression, of heart failure has great allure. However, although conceptually appealing, the promise of cell therapy is so far unfulfilled.

The state of the art

Multiple cell therapy approaches for heart disease have been tested in a clinical setting over the years (Fig. 2). The first systematic efforts in cardiac regeneration, which occurred by the turn of the millenium⁴, were based on the much earlier finding that autologous skeletal myoblasts can engraft and proliferate when transplanted into the heart⁵. Skeletal muscle, unlike cardiac muscle, is not coupled to the surrounding syncytium, nor does it beat spontaneously. Nevertheless, the hope was that the transplant would trigger the formation of new contractile units within the myocardium to boost contraction. The research and development programme followed a logical sequence, starting with small animal models⁶, continuing to more realistic preclinical models⁷ and, ultimately, running patient trials. Clinical testing of surgically implanted skeletal myoblasts in patients with heart failure showed hints of efficacy but also enhanced arrhythmogenesis⁸; consequently, development efforts for this cell type seem to have been abandoned.

As the skeletal myoblast approach was being tested, a less methodical translational programme unfolded around the study of bone-marrow-derived cells for acute myocardial infarction (AMI). In 2001, researchers made the extraordinary claim that “locally delivered bone marrow cells can generate de novo myocardium, ameliorating the outcome of coronary artery disease”⁹. This discovery in a mouse model of AMI was subsequently discredited¹⁰, but despite this clinical studies followed almost immediately¹¹. The general rationale for the therapy was as follows: patients presenting with AMI underwent routine clinical care, consisting of percutaneous coronary intervention to re-open the occluded coronary artery; afterwards (typically 1–14 days after the AMI), bone marrow aspiration was performed, and autologous bone marrow mononuclear cells were isolated and delivered by intracoronary infusion into the injured region of the heart. Several thousand such patients underwent the procedure¹². The general treatment scheme has proven to be quite safe, but overall efficacy remains uncertain. With the possible exception of the ongoing BAMI trial¹³, no study has been adequately powered to assess hard clinical outcomes such as rehospitalization and mortality. Meta-analyses resulted in conflicting conclusions — some showed improvement¹⁴, but others revealed no clear benefit^{12,15}. Overall, no convincing evidence of heart regeneration has been presented in any of the clinical studies involving bone marrow mononuclear cells.

While trials of bone marrow cells were proceeding, experimentation began with second-generation cell products. Some of these, notably bone-marrow-derived mesenchymal stem cells (MSCs)¹⁶, were adapted from other indications for which they had been therapeutic candidates for some time. Variants of MSCs that have reached clinical testing for heart failure include cardiopoietic cells (autologous MSCs fortified in vitro¹⁷) and antigen-selected mesenchymal precursor cells¹⁸. Regular MSCs have been used primarily in small, uncontrolled trials¹⁹, yet cardiopoietic cells²⁰ and mesenchymal precursor cells²¹ have been tested more rigorously. Other cell sources have also been the focus of research: for instance, endothelial progenitor cells and autologous circulating bone-marrow-derived cells selected

on the basis of CD34 expression seem to be effective in mitigating refractory angina²². Furthermore, alternative strategies were explored for recruiting and augmenting innate regenerative responses in the heart. In this context, cardiac progenitor cells seemed particularly attractive, owing to their potential to engraft, proliferate and differentiate into new myocardium. Heart cells selected for expression of the c-kit surface antigen were proposed to work by these canonical mechanisms²³, which motivated the SCIPIO trial of autologous c-kit-selected heart cells²⁴. Unfortunately, once again the background science supporting this trial was later called into question²⁵, and the SCIPIO trial itself triggered an editorial expression of concern in the *Lancet*²⁶.

The cell types used in these studies were, at best, adult progenitor cells and not pluripotent stem cells. Although pluripotent cells are more likely to work via the originally hypothesized canonical mechanisms, they have experienced a glacially slow translational-development trajectory. Since 1998, human pluripotent cells have been known to produce heart tissue in vitro and in vivo²⁷. Nevertheless, clinically relevant studies injecting pluripotent-cell-derived cardiomyocytes (or cardiomyocyte precursors) in large-animal AMI models have uncovered safety concerns in the form of severe ventricular arrhythmias, which were detected frequently^{28,29}. Such arrhythmias could be fatal in humans; therefore, clinical studies with intramyocardially injected pluripotent-cell-derived cardiomyocytes will be difficult to justify until this complication is avoided. Still, some researchers have leapfrogged further study and transplanted embryonic stem-cell-derived cardiomyocyte sheets onto the surface of the heart (that is, epicardially) in several patients with advanced heart failure. The cases reported to date have revealed no apparent safety concerns³⁰. However, unlike cells that are injected into the heart muscle itself, epicardially administered cells are unlikely to work by engrafting and proliferating to create new heart muscle³¹, thus falling short of the conceptual attractiveness of pluripotency — that is, the ability of stem cells to self-renew, with potential differentiation into functional cardiomyocytes.

Several major clinical trials of cell therapy for heart disease are currently recruiting (Table 1). At the moment, only three cell types seem to be in active commercial development as cell-based therapies with a view to ultimate product registration for cardiac indications: autologous bone marrow mononuclear cells (CardiAMP trial, [NCT02438306](#)) and allogeneic mesenchymal precursor cells (DREAM HF-1 trial, [NCT02032004](#)) are both in phase-III testing for heart failure with reduced ejection fraction, and allogeneic cardiosphere-derived cells (CDCs) are being developed for various specialized types of heart failure, notably the cardiomyopathy associated with Duchenne muscular dystrophy (HOPE-2 trial, [NCT03406780](#)).

Barriers to translation

The field of cardiac cell therapy has not lacked ideas, many of which have undergone extensive preclinical testing to establish proof of concept. How then, can one rationalize the inability, to date, to translate any of those concepts to approved products? Various obstacles to translation, depicted schematically in Fig. 3, are discussed in detail below.

Legacy obstacles.

Multiple translational failures, compounded by allegations of scientific irregularities surrounding reports of bone-marrow-derived cells and c-kit-selected heart cells, have poisoned the public perception of cardiac cell therapy. Clinical studies have often proceeded with little information regarding cell dosing, a parameter that is rarely optimized in translational studies prior to clinical application. Likewise, the risk–benefit ratio of the various delivery options receives little attention before clinical studies are initiated. This gloomy perspective has been compounded by hype — expectations are raised only to be dashed later, creating a state of confusion that undermines the entire field. As a case in point, a recent Editorial³², published in *Nature Biotechnology*, that targeted cardiologists' hype^{33,34} over a trial of CD34-selected cells for AMI³⁵ ended up as a wholesale indictment of cardiac cell therapy. Given these considerations, it is no wonder that the investment climate for cardiac cell therapy is lukewarm at best, and that companies with significant heart-regeneration programmes tend to fare poorly in the public markets. Without commercial interest, it is impossible to move from carefully curated proof-of-concept academic studies to larger placebo-controlled trials and, eventually, to approved products.

Manufacturing challenges.

Although there is ample evidence for the efficacy of cardiac cell therapy in preclinical models³⁶, the fact that cells are fragile living entities that can be difficult to manufacture and to handle precludes the application of standard manufacturing processes³⁷. Cellular therapeutic candidates generally originate in academic laboratories, where they are made to research-grade standards and tested pre-clinically without much attention to scalability or regulatory compliance. First-in-human studies require relatively modest manufacturing process optimization and, because they are designed to treat only a handful of patients, the scalability, cost and reproducibility of the cell product are secondary concerns. Only a few cell sources have progressed beyond phase-I clinical trials of cell therapies for cardiovascular indications, and the most perilous pitfalls lie in the steps that follow those first trials. As a product undergoes further development, the typical translational sequence requires discovery scientists to hand over to biotechnology companies or to contract manufacturing operations. There, an oft-heard mantra is 'good management trumps good science': cost of goods sold, process development and quality control — terms well-known in the pharmaceutical industry by their acronyms (COGS, PD and QC, respectively), but alien to the discovery scientist — begin to dominate manufacturing priorities. Unlike small molecules, whose synthesis and standards for purity and formulation are well-established, cells are alive and dynamic, evolving and adapting in response to the environment. Consequently, maintaining efficacy is a continuing challenge, especially for cell types that undergo senescence (or lose potency) after multiple passages following isolation from source tissues³⁸. Importantly, the optimization of manufacturing processes (for instance, a switch from monolayer culture to suspension culture) introduces changes that may seem innocuous, but can fundamentally alter the final product, often in unpredictable ways³⁹. Cryopreservation of allogeneic cells for increased shelf-life further alters the product, with potential diminished efficacy after thawing. In the extreme, the therapeutic candidates can stray so far from their intended formulations that they lose disease-modifying properties. The risk of product drift is influenced by the prevailing corporate culture; without strong

scientifically based management, bioactivity takes a back seat to process expediency, potentially derailing a cell product⁴⁰.

Reproducibility and efficacy.

A major concern for commercial biomedical products is consistency. To conform to regulatory standards, a product sold today must be comparable in identity, potency and composition to the same product manufactured and sold years from now. Sequential quality-control procedures must therefore be put in place at various steps in the manufacturing process to validate the equivalence of all cell banks. There is no guide book for how this should be done, as no allogeneic cell therapy products have been approved in the United States (with the exception of GINTUIT, an allogeneic keratinocyte and fibroblast product approved by the US Food and Drug Administration in 2012 for topical use in dental applications). It seems reasonable to presume that the more attention paid to product uniformity and quality, the greater the risk of product drift. A related, significant concern in commercial product development is diminished efficacy, which should be tackled by the establishment and validation of assays that measure potency. This can be a challenging endeavour, as cell therapy has such diverse targets that it can be difficult to pinpoint a single molecule or pathway as being particularly essential for therapeutic efficacy. In vivo models, which prevail at the discovery stage, have the virtue of providing integrative, functional readouts, but they suffer from biological variability, long duration, high cost and subjectivity of interpretation. Reductionist models in vitro, on the other hand, can fall short in terms of disease relevance. Rapid, reliable, indication-relevant potency assays are highly desirable but rarely implemented. As an example of such an assay, secretion of tumour necrosis factor- α receptor serves as a potency marker for Prochymal, an allogeneic MSC product approved in Canada for graft-versus-host disease (this receptor binds a pro-inflammatory cytokine that is linked mechanistically to graft-versus-host disease⁴¹). Insofar as cells are viewed as black boxes, progress towards better potency assays will be elusive. Therefore, detailed mechanistic dissection is a route to identifying determinants of efficacy that, in turn, can reveal markers of potency.

Safety.

Cell therapies carry unique safety-related concerns. These can generally be categorized as either related to delivery strategies or as specific to the cells themselves. Delivery methods used to date in the clinic vary from innocuous to highly invasive, with accordingly escalating risk. When targeting the heart, intravenous infusion (the least extreme method), has been assumed to be generally inferior, owing to the lack of direct administration to the heart itself; however, intravenous infusion is poised for a resurgence, given the increasing evidence that cells can exert long-distance effects mediated by exosomes (as reviewed below).

Most clinical trials to date, however, have presumed that direct delivery is required. Catheters have frequently been used to infuse cells into the coronary circulation, with a favourable safety profile comparable to that of routine coronary angiography (which results in serious complications in approximately 1 in 500 patients⁴²). The coronary route has the advantage that cells are administered into a natural space (the coronary arterial lumen), where they can lodge and eventually extravasate⁴³; no artificial space is created, nor is the

heart disrupted mechanically. The recent demonstration that stop-flow vessel occlusion is not required for efficacy has further simplified intracoronary delivery^{44,45}. The use of experimental transendocardial intramyocardial injection catheters is the riskiest method by far, and is only supported by a paucity of reliable data⁴⁶. In this method, the goal is to introduce remotely deployed needles into multiple sites in the heart, thereby enabling the injection of the cell product into the myocardium itself. Along with the creation of an unnatural space, and tissue disruption, the method has a considerable delivery-related mortality, driven by a high risk (from approximately 1 in 25 (ref. ²²) to 1 in 80 (ref. ⁴⁷)) of iatrogenic cardiac perforation when applied to patients. Nevertheless, several clinical trials continue to use intramyocardial injection (of the seven ongoing trials listed in Table 1, three are using this poorly validated method). In addition, open-chest surgery has been used to inject cells directly into the heart⁴⁸, or to apply cell-containing sheets or gels to the surface of the heart (epicardial administration^{30,49}). Surgical delivery methods are so invasive, however, that they are justifiable only in patients with other indications for cardiac surgery.

When considering risks associated with the cells themselves, the risk of neoplasm is ever-present with proliferating transplanted cells. Pluripotent cells can form tumours *in vivo*; therefore, pre-differentiation to the desired lineage, and verification of absence of lingering pluripotent cells, are vital precautions in contemplating their clinical application⁵⁰. This risk is more than theoretical, as tumours have been reported in several patients treated in commercial stem cell clinics^{51–53}. Allogeneic adult stem cells, which are likely to be cleared immunologically over time⁵⁴, may offer an added measure of safety in this regard, but immune rejection may occur so quickly that it undermines the ability of allogeneic cells to exert paracrine mechanisms of action, ultimately limiting efficacy⁵⁵. Another potential safety concern with transplanted cells is the chance for progressive sensitization to foreign antigens, which might lead, at least in principle, to faster clearance of allogeneic cells in repeat-dosing protocols⁵⁵. This may become a problem in regimens that depend on multiple cell doses, applied sequentially over time, as is likely to be required for sustained efficacy in diseases with a progressive underlying pathology such as Duchenne muscular dystrophy. Crossmatching — that is, determining compatibility between donor and recipient — may mitigate the theoretical concern of sensitization, if it turns out to be genuine⁵⁶.

Another safety consideration, unique to cardiac applications of cell therapy, is the potential for enhanced arrhythmogenesis. The heart functions as an electrical syncytium in which the transmission of electrical impulses relies on the efficient coupling between excitable myocardial cells. Transplanted cells can disrupt the electrical impulses, either by creating uncoupled or poorly coupled islands of new tissue that block conduction (thus potentially functioning as parasystolic foci favouring abnormal automaticity), or by coupling with surrounding myocardium and influencing local activation and repolarization⁵⁷. Skeletal myoblasts worsen arrhythmia by the first mechanism (conduction block), an effect that may be mitigated by the use of epicardial cell sheets⁵⁸. By contrast, immature cardiomyocytes derived from pluripotent cells cause arrhythmias by coupling with surrounding mature heart cells, leading to regional inhomogeneities that facilitate re-entry or triggered activity⁵⁷. In this context, non-integrating adult heart cells, working by paracrine mechanisms, may have a safety advantage over engrafting cell types.

Mechanistic insights for overcoming translational barriers

Much of the work in the development of cell therapies to date has been driven more by doctrine than by a deep mechanistic understanding of precisely how cells exert therapeutic benefits. This may underlie the general failure to advance preclinical findings and small-scale clinical trials. For cell therapies to succeed in the clinic, they need to be centred on the underlying biological mechanisms. Mechanistic insights are leading the field away from the cells themselves and refocusing on the cells' paracrine functions, and will ultimately zone in on defined factors. Understanding the defined factors, in turn, can inform the clever use of cells as therapeutic candidates. The development trajectory of CDCs for heart failure, for instance, exemplifies how mechanistic discoveries can feed-back on and focus the translational process of cell therapies. Such a trajectory is only possible by discarding doctrine and following the data.

The promise of CDCs.

In 2004, my colleagues and I began to develop CDCs as therapeutic candidates for heart failure, and in 2007 we reported a manufacturing process for CDCs and their properties⁵⁹ (Fig. 4). Since then, about 160 papers have been published using this cell type from over 45 independent laboratories worldwide, confirming the cells' therapeutic bioactivity⁶⁰. CDCs uniformly express CD105 and are negative for CD45 and other haematopoietic markers; they qualify as cardiac progenitor cells, being of intrinsic cardiac origin⁶¹, multipotent and clonogenic⁶². On the basis of these features, the phase-I clinical trial CADUCEUS (NCT00893360) used autologous cells⁶³ with the presumption that transplanted cells would generate new tissue that would be rejected immunologically if it were not a perfect match to the host. CADUCEUS showed autologous CDCs to be safe, and yielded hints of regenerative efficacy. Three other trials of autologous CDCs have been conducted independently in Japan, in infants with hypoplastic left heart syndrome and in adult heart failure, with no apparent safety concerns and multiple signs of efficacy^{49,64,65}.

From autologous to allogeneic cells.

There is extensive evidence that cell therapy works indirectly, even with bona fide progenitor cells (Fig. 5). Whereas this is true in general, the evidence is particularly strong for CDCs. Few (< 1% of injected) cells are detectable 3–4 weeks after transplantation, but functional and structural benefits persist for at least 6 months^{54,66}. During the approximate 2 weeks in which appreciable numbers of transplanted CDCs persist in the tissue, the cells indirectly induce cardiomyogenesis in the host myocardium⁶⁷. Notably, the bioactivity of CDCs is not limited to fostering regeneration; the cells also induce antifibrotic, anti-inflammatory and immunomodulatory effects⁶⁸, harnessing all of the benefits (identified in Fig. 1) that are critical to improve heart structure and function. Attempts to pinpoint the key paracrine signals initially focused on growth factors and chemokines, such as SDF-1 (ref. ⁶⁶).

The realization that transplanted cells do not linger in the heart for long, yet exert lasting benefits, motivated the testing of cells from unrelated donors without immunosuppression. Preclinical studies showed that allogeneic CDCs are safe, and that they are as effective as autologous CDCs^{54,69}, even with repeated dosing⁷⁰. This has led to three completed

(DYNAMIC⁷¹; ALLSTAR, [NCT01458405](#); and HOPE-Duchenne, [NCT02485938](#)) and three ongoing (ALPHA for pulmonary hypertension, [NCT03145298](#); Regress-HFpEF for heart failure with reduced ejection fraction, [NCT02941705](#); and HOPE-2 for Duchenne muscular dystrophy, [NCT03406780](#)) trials of allogeneic CDCs. The emphasis of cell therapy research shifted in 2014, when exosomes were first implicated as the mediators of CDC benefits⁷².

The promise of exosomes.

Serum-free medium conditioned by CDCs contains extracellular vesicles, including exosomes, as gauged by particle size and payload (Fig. 6a). Exosomes are products of the ceramide-requiring endolysosomal pathway, and arise from the fusion of surface membrane invaginations (endosomes) and products of the Golgi apparatus to create multivesicular bodies⁷³. Accordingly, inhibitors of ceramide synthesis, such as GW4869, block the secretion of exosomes. These nanoparticles are secreted not only by CDCs but by all other eukaryotic cells examined to date, and contain rich repertoires of bioactive nucleic acids and proteins that can be taken up by, and transferred to, neighbouring or distant cells. In a mouse model of AMI, blocking exosome production renders CDCs ineffective (Fig. 6b), whereas CDC exosomes (CDC_{exo}) can reproduce the benefits of the parent CDCs (Fig. 6c)⁷². Thus, exosomes are necessary and sufficient for CDC efficacy. The concepts described here for heart-derived progenitor cells have since been generalized to all other cell types under consideration as therapeutic candidates for heart disease; even pluripotent-stem-cell products are now acknowledged to act largely via exosome secretion^{74–76}. Many, if not most, of the effects of exosomes are mediated by their RNA contents, specifically miRs and other non-coding RNAs (ncRNAs)⁷⁷. Among ncRNAs, non-miR Y RNA fragments are richly expressed in CDC_{exo} and modulate target cell gene expression⁷⁷. The emerging concept for the mechanism of action of CDC_{exo} relies on progenitor cells secreting exosomes that transfer payloads into target cells, inducing transcriptomic and phenotypic changes that underlie the benefits of cell therapy. As suggested by the data (Fig. 6c), exosomes may themselves be attractive next-generation cell-free therapeutic candidates.

Will exosomes supplant cells entirely? Probably not. Cells have been in therapeutic development for decades, whereas purified exosomes have undergone very limited testing in humans, and never for cardiac indications⁷⁸. The challenge is in identifying the situations in which cells are preferable to exosomes. Exogenously delivered exosomes are still poorly understood in terms of biodistribution, delivery and bioactivity in vivo; they can travel near and far⁷⁹, although uptake is stronger and most predictable with local administration within tissue. Perhaps unsurprisingly, cells may turn out to be the best vehicles to deliver exosomes and their contents to target tissues in certain situations. AMI is one indication in which cells seem to be more efficacious than exosomes, at least when both products are administered via the intracoronary route⁸⁰, as cells lodge within the microvasculature and function as sustained-release vehicles for exosomes, whereas administered exosomes pass quickly through the coronary vessels into the rest of the body, diluting their efficacy.

Another instance in which cells trump exosomes arises when cells actually engraft meaningfully within the tissue and work canonically. Pluripotent-cell-derived

cardiomyocytes or cardiomyocyte precursors have been shown to be capable of long-term engraftment⁸¹, although the efficiency of the process may be forbiddingly low. Engraftment and differentiation of transplanted cells also exacerbate the risk of arrhythmia, a potentially lethal complication^{28,29}. Disease targets in which pluripotent-cell-derived cardiomyocytes may be preferable include structural defects in the heart associated with localized deficiency of muscle tissue, such as ventricular septal defects or ventricular aneurysms. Here, cell patches grown ex vivo and implanted surgically or percutaneously may find focused application. Such patches may also function adequately without requiring full electromechanical integration with surrounding heart tissue, conceivably minimizing the risk of arrhythmia. From an electrophysiological perspective, the most hazardous applications of pluripotent-cell-derived cardiomyocytes are those involving intramyocardial delivery of dispersed cells into regions of infarcted tissue, where risk may well outweigh benefit⁵⁷.

From cells to exosomes to soluble factors

Going forward, it is important to identify specific situations in which cell therapy may be preferable to therapy using downstream mediators; otherwise, cell therapy is arguably an interim technology. Once it became clear that some effects of cell therapies are indirect, the goal logically shifted to understanding and optimizing the mediators, so they themselves could be used as therapeutic candidates. As reviewed previously⁷⁸, exosomes offer the potential to overcome key limitations of cell therapy. Potential advantages over cells include product stability⁸², even with repeated freeze–thaw cycles⁸³, and immune tolerance (human CDC_{exo} are effective in non-immunosuppressed mice⁸⁴, rats⁷⁷ and pigs⁸⁰, even following repeated administration⁸⁴). Exosomes are efficacious after systemic delivery because they are relatively non-immunogenic^{84–86}, and their dosing is not limited by microvascular plugging or by loss of transplanted cell viability⁷⁷. Moreover, there are multiple approaches to enhancing exosome efficacy, including genetic engineering of the parent cells⁸⁷. Commercial product development for exosomes is, however, still in its infancy, so expectations for the pace of progress should be cautious.

When the exploration of cell therapy applications for heart disease began, there was no inkling that exosomes were involved. Now that their role is clear, the focus has moved to pinpointing the factors within exosomes that are vital to conferring efficacy in any given setting. In AMI, for example, certain miRs (miR-146a, ref. ⁷²; and miR-181b, ref. ⁸⁸) and a Y RNA fragment⁷⁷ have been implicated as exosomal contents that are central for cardioprotection (Fig. 7). One implication of these findings is clear: the defined factors themselves, individually or in combination, can become new therapeutic candidates. Another implication of these observations is the potential to use mechanistic insights to feed-back on the development process of cell therapies towards their improvement. For instance, mechanistically based potency assays could be developed around the quantification of the identified factors in exosomes secreted by a cell therapy candidate. Thus, mechanistic insight, instead of simply leading us away from cells towards exosomes and defined factors, should lead to improvements in the quality, reproducibility and efficacy of parent-cell-based therapeutic approaches. Nevertheless, major gaps remain regarding the comprehension of mechanisms, and additional fundamental research is necessary to refine and inform the development of rational and robust potency assays for cell therapy.

Outlook

The mechanistic deconstruction of cell therapy has led the way from autologous cell therapy, which was based on canonical doctrine, to exosomes and their contents as next-generation therapies. This dramatic evolution has played out over less than a decade, with CDCs offering a case study of the key mechanistic discoveries: (1) in 2004, the isolation of CDCs from the human heart, for use in autologous therapy; (2) in 2014, the recognition of durable therapeutic benefits despite cell transience — this led to the allogeneic paradigm in 2012; (3) in 2014, the identification of exosomes as mediators, which has implications for cell-free therapeutics; and (4) since 2014, the mining of exosome contents has helped identify defined factors that may usher in next-generation therapeutics. The identification of exosomes as mediators resulted from the recognition of the equivalent benefits of autologous cell sources and allogeneic cell sources. The mining of exosome contents, in turn, pinpointed certain ncRNA species as defined factors worthy of attention in their own right.

Despite the progression away from cells towards defined factors, cell-based therapies retain their potential for application in defined indications. For adult cell therapy applications, enhancing and capitalizing on the indirect effects of cells will be central to maximizing their efficacy. For pluripotent-derived cells, the emphasis should logically be on improving engraftment and maximizing the adult phenotype to mitigate arrhythmic risk in applications to treat myocardial infarction. Beyond their roles as candidates for novel therapeutic approaches, cells have already begun to pay off as discovery platforms; in the process of investigating mechanisms, the potential value of exosomes and their contents has come into focus. Recognition of the critical bioactive secreted factors in any given setting provides a mechanistic rationale for the development of new potency assays. Such assays, in turn, have the potential to improve cell therapy by providing reliable beacons of disease-modifying bioactivity, as checks and balances against the product drift that can be introduced by commercially optimized manufacturing processes and quality-control efforts.

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Fig. 1 |. Biological processes modulated by cell therapy.

The direct progeny of transplanted cells can generate new heart muscle and blood vessels by canonical mechanisms. Yet other biological processes may be stimulated or suppressed via non-canonical (indirect) mechanisms of cell action.

Fig. 2 |. Clinical testing of cell therapies for heart disease.

Cell types that are actively being studied are depicted as boxes with an open righthand edge. Cell types in fully enclosed boxes represent programmes that no longer seem in active clinical development since the time of the last reported trial. The thickness of the triangles is roughly proportional to the number of trials conducted at each time point; phase-I trials are depicted in blue, and phase-II and later trials in red. ESCs, embryonic stem cells.

Fig. 3 |. Obstacles in the translation of cell therapy, from proof of concept through to product approval.

The process is akin to navigating a maze, with obstacles including legacy concerns introduced by a history of unsound scientific practices and relentless hype; the transition from small-scale phase-I manufacturing to commercial-scale manufacturing introduces the potential for product drift, lack of reproducibility of key product characteristics, difficulties establishing mechanistically based potency assays, and concerns related to safety.

Fig. 4 |. CDC properties.

The approach for specimen processing for CDC growth and expansion. Figure adapted from ref. ⁵⁹, Wolters Kluwer Health, Inc.

Fig. 5 |. Changes from canonical to indirect (paracrine) mechanisms of action of cell therapy. Diagram based on evidence provided in refs ^{54,66,69,89–92}.

Fig. 6 |. Exosome biology and evidence of exosome efficacy.

a, Schematic of exosome biogenesis and release. (1) Exosome payload, including proteins and RNA, are synthesized in the nucleus and matured in the endoplasmic reticulum. (2) Sorting occurs in the trans-Golgi system (shown in red), where exosome signals are labelled with ceramide and with trafficking proteins that mediate budding into the endosome. (2') The endosome itself is a product of invagination of the plasma membrane. Intraluminal vesicles form by fusion with the endosome, and acquire surface-marker profiles that are conserved. The markers include tetraspanins and heat-shock proteins, as well as markers specific to the parent cell. (3) The multivesicular body progressively acidifies, which leads to fusion with the plasma membrane and the release of intraluminal vesicles (exosomes). nSMase, neutral sphingomyelinase. **b**, Blockade of CDC exosome biosynthesis with the small molecule GW4869 undermines CDC efficacy in improving heart function after myocardial infarction (MI). **c**, CDC exosomes mimic the functional benefits of CDCs after MI. LVEF, left ventricular ejection fraction. Panels **b** and **c** adapted from ref. ⁷², Elsevier.

Fig. 7 |. Defined exosome contents implicated in cdc-mediated cardioprotection.

Left: biogenesis and secretion of exosomes. Right: exosome contents. lncRNA, long non-coding RNA; miRNA, microRNA; IL-10, interleukin 10; PKCδ, protein kinase C isoform; TRAF-6, tumour necrosis factor receptor- associated factor 6. Figure adapted from ref. ⁷⁸, Elsevier.

Table 1 |

Clinical trials of cell therapy for heart disease

Trial name	Indication	Design; sponsor; phase	Number of patients projected	Delivery method
Bone marrow mononuclear cells				
REPEAT (NCT01693042)	Chronic post-infarction heart failure	Autologous cells, single versus dual infusion; academic; phase II–III	676	Intracoronary
CardiAMP Heart Failure Trial (NCT02438306)	Chronic post-infarction heart failure	Autologous cells, randomized with sham control; BioCardia; phase III	250	Transendocardial intramyocardial delivery
Andalucia DCM Trial (NCT02033278)	Dilated cardiomyopathy	Autologous cells, DBPCT; academic; phase II–III	51	Intracoronary
BAMI (NCT01569178)	Acute myocardial infarction	Autologous cells, randomized to cell infusion or standard care; academic; phase III	350	Intracoronary
Mesenchymal cells				
DREAM HF-1 (NCT02032004)	Chronic heart failure with reduced ejection fraction	Allogeneic MPCs, randomized with sham control; Mesoblast; phase III	600	Transendocardial intramyocardial delivery
Cardiosphere-derived cells				
HOPE-2 (NCT03406780)	Cardiomyopathy and skeletal myopathy of Duchenne muscular dystrophy	Allogeneic, multiple repetitive doses, DBPCT; Capricor; phase III	80	Intravenous
Combinations				
CONCERT-HF (NCT02501811)	Ischaemic cardiomyopathy	Autologous bone marrow MSCs and/or autologous c-kit-selected heart cells, DBPCT; academic; phase II	144	Transendocardial intramyocardial delivery

Actively recruiting multicentre trials were taken from <https://clinicaltrials.gov> (accessed 22 February 2018). Each trial is listed with its National Clinical Trial identifier. DBPCT, double-blind placebo- controlled trial; MPC, mesenchymal precursor cell; MSC, mesenchymal stem cell.