

Cardiac cell therapy: where we've been, where we are, and where we should be headed

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Introduction: Stem cell therapy has emerged as a promising strategy for the treatment of ischemic cardiomyopathy.

Sources of data: Multiple candidate cell types have been used in preclinical animal models and in clinical trials to repair or regenerate the injured heart either directly (through formation of new transplanted tissue) or indirectly (through paracrine effects activating endogenous regeneration).

Areas of agreement: (i) Clinical trials examining the safety and efficacy of bone marrow derived cells in patients with heart disease are promising, but results leave much room for improvement. (ii) The safety profile has been quite favorable. (iii) Efficacy has been inconsistent and, overall, modest. (iv) Tissue retention of cells after delivery into the heart is disappointingly low. (v) The beneficial effects of adult stem cell therapy are predominantly mediated by indirect paracrine mechanisms.

Areas of controversy: The cardiogenic potential of bone marrow-derived cells, the mechanism whereby small numbers of poorly-retained cells translate to measurable clinical benefit, and the overall impact on clinical outcomes are hotly debated.

Growing points/areas timely for developing research: This overview of the field leaves us with cautious optimism, while motivating a search for more effective delivery methods, better strategies to boost cell engraftment, more apt patient populations, safe and effective 'off the shelf' cell products and more potent cell types.

Keywords: stem cell therapy/cardiac stem cells/heart regeneration

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Introduction

Cardiovascular disease remains the leading cause of death and disability in Americans, claiming more lives each year than cancer, diabetes mellitus, HIV and accidents combined.¹ Ischemic heart disease is the predominant contributor to cardiovascular morbidity and mortality; ~1 million myocardial infarctions (MIs) occur per year in the USA, while ~5 million patients suffer from chronic heart failure.² Death rates have improved dramatically over the last four decades,³ but new approaches are nevertheless urgently needed for those patients who go on to develop ventricular dysfunction.⁴ Over the past decade, stem cell transplantation has emerged as a promising therapeutic strategy for acute or chronic ischemic cardiomyopathy. Multiple candidate cell types have been used in preclinical animal models and in humans to repair or regenerate the injured heart either directly or indirectly (through paracrine effects), including: embryonic stem cells (ESCs),^{5–7} induced pluripotent stem cells (iPSCs),⁸ neonatal cardiomyocytes,^{9,10} skeletal myoblasts (SKMs),¹¹ endothelial progenitor cells (EPCs),¹² bone marrow mononuclear cells (BMMNCs),^{13–15} mesenchymal stem cells (MSCs)¹⁶ and most recently cardiac stem cells (CSCs).^{17,18}

Although no consensus has yet emerged, the ideal cell type for the treatment of heart disease should:

- (i) be safe, i.e. not create tumors (a very real possibility that has been observed after the delivery of undifferentiated ESCs or iPSCs to the heart¹⁹) or arrhythmias (a well-documented risk of SKM transplantation^{20–22});
- (ii) improve heart function;
- (iii) create healthy and functional cardiac muscle and vasculature, integrated into the host tissue;
- (iv) be amenable to delivery by minimally-invasive clinical methods;
- (v) be ‘off the shelf’ available as a standardized reagent;
- (vi) be tolerated by the immune system; and
- (vii) circumvent societal ethical concerns.

At present, it is not clear whether such a ‘perfect’ stem cell exists; what is apparent, however, is that some cell types are more promising than others.

In this brief review, we provide a critical assessment of the various cell types used for heart regeneration, discuss the areas of agreement and controversy arising from the first generation of clinical trials, and touch upon the future directions of cell therapy for heart disease. The focus of this brief review is on cells that are already in the clinic, or soon will be. Thus, the treatment of iPS and ES cells is intentionally cursory. The reader is referred elsewhere for reviews on these topics.^{23,24}

Embryonic stem cells/induced pluripotent stem cells

ESCs, derived from the inner mass of the developing embryo in the blastocyst stage, are the prototypical stem cells. They have the capacity of self-renewal, can be clonally expanded and are capable of differentiating into any cell type in the body, including cardiomyocytes.^{23,25} However, significant obstacles severely limit their clinical translatability. First, their unlimited differentiation potential is a double-edged sword; when these cells are transplanted in their primitive undifferentiated state, they form teratomas,¹⁹ benign tumors derived from all three germ layers (the endoderm, ectoderm and mesoderm). Second, due to their allogeneic origin, they carry the risk of immune rejection; there is now clear evidence that the differentiated progeny of ESCs are rejected by the host immune system¹⁹; under certain applications requiring only temporary engraftment, however, rejection of transplanted cells may be a virtue. Finally, ESCs are ethically problematic since they are created from early human embryos (discarded after *in vitro* fertilization).²⁶ Despite these shortcomings, the first clinical trial with cells derived from allogeneic ESCs has commenced in the USA, in patients with spinal cord injury.²⁷

During the last 5 years, remarkable advances have been made generating pluripotent embryonic-like stem cells from somatic (adult) cells (e.g. dermal fibroblasts), through the introduction of four genes via retroviruses.²⁸ The resultant iPSCs closely resemble ESCs and can be subsequently directed/guided to differentiate into desirable specific cell types. These revolutionary techniques make the possibility of patient-specific pluripotent cells an imaginable reality and provide an alternative source for cardiogenic cell lines; functional cardiomyocytes have now been successfully derived from both mouse²⁹ and human iPSCs.³⁰ As exciting as these approaches may be, significant roadblocks [risk of teratoma formation associated with the pluripotent state, time required to derive and characterize iPSCs from any given patient (~4 months), low efficiency of cardiogenic differentiation, genetic abnormalities and high cost^{31,32}] preclude short-term clinical applicability. Methods to expedite the generation of cardiomyocytes from non-contractile somatic cells, without transit through a pluripotent state, are intriguing.^{33,34} Nevertheless, the use of genetically-modified cells which have undergone nuclear reprogramming will face significant regulatory hurdles before clinical applications commence.

Skeletal myoblasts

Skeletal myoblasts (SKMs) are conceptually attractive for cellular cardiomyoplasty: they have a contractile phenotype, can be harvested

for autologous transplantation, and are resistant to ischemia. After a decade of experimental studies,^{35–37} SKMs were the first cell type to enter the clinical arena for heart regeneration. In June 2000 autologous SKMs, isolated and expanded from a thigh muscle biopsy, were intramyocardially injected in a patient with severe ischemic heart failure as an adjunct to coronary bypass grafting (CABG) surgery.³⁸ Several small non-randomized phase I trials ensued demonstrating a functional benefit, albeit with a high incidence of ventricular arrhythmias.^{20,21,39} SKMs differentiate into multinucleated myotubes (not cardiomyocytes) after injection into the heart. These myotubes lack gap junctions and form islands of conduction block in the heart, resulting in electrical inhomogeneities that slow conduction velocity and predispose to reentrant ventricular arrhythmias.⁴⁰ In sharp contrast to the functional benefit observed in the early uncontrolled studies, the first prospective randomized placebo-controlled phase II SKM trial (MAGIC trial), exhibited lack of efficacy and was discontinued prematurely.¹¹ In addition, despite the use of prophylactic amiodarone, a trend towards excess arrhythmias was observed in myoblast-treated patients, thus confirming the safety concern that had already been raised by earlier phase 1 trials. On the other hand, the recently-published SEISMIC trial⁴¹ argued that injection of autologous SKMs in HF patients is safe and may provide symptomatic relief (a trend towards increased exercise tolerance was observed in the cell-treated group); nevertheless, no significant effect on global LVEF was detected. Taken together, the trajectory of SKMs is instructive and argues against premature enthusiasm solely on the basis of preclinical studies.

Bone marrow-derived cells

Unlike SKMs, bone marrow-derived cells moved into patients without the benefit of a convincing preclinical development program; in fact, the first report of clinical application of bone marrow-derived cells for heart regeneration⁴² surfaced within 4 months of the publication of a rodent study showing extensive engraftment and cardiogenic differentiation of bone marrow-derived cells in mice.⁴³ Clinical application was catalyzed by the relative accessibility of bone marrow, the large numbers of unfractionated autologous cells that can be obtained without *ex vivo* expansion, and the extensive clinical experience with bone marrow transplantation. Ironically, the initial report of extensive transdifferentiation of marrow-derived cells into cardiomyocytes has proven to be controversial, in that several laboratories have been

unable to reproduce the findings.^{44,45} Nevertheless, clinical studies have continued apace.

The bone marrow is a highly heterogeneous tissue, containing several different cell populations including rare hematopoietic, endothelial and mesenchymal stem cells. Human hematopoietic stem cells (HSCs) can traditionally be defined as rare CD34+ cells capable of reconstituting all blood lineages⁴⁶ and, possibly, the ability to transdifferentiate into cardiomyocytes, endothelial cells and smooth muscle cells *in vivo*.⁴⁷ EPCs are a subset of hematopoietic cells that promote neovascularization either directly (differentiation into endothelial cells)⁴⁸ or indirectly (secretion of pro-angiogenic cytokines).⁴⁹ MSCs can be roughly defined as CD105 + CD90+ cells, isolated by preferential adherence to plastic in tissue culture, which are capable of osteogenic, chondrogenic and adipogenic differentiation.⁵⁰ MSCs purportedly exhibit low immunogenicity,⁵¹ rendering allogeneic applications plausible. It should be noted that BMMNCs, isolated by density centrifugation following bone marrow aspiration, actually contain very few stem cells (~2–4% HSCs/EPCs and ~0.01% MSCs); the vast majority of BMMNCs comprise committed hematopoietic cells at various stages of maturation.⁵²

In the clinical setting, autologous BMMNCs are by far the most frequently used cell type for treatment of acute MI. To date, >1000 patients have been treated with bone marrow-derived cells (either unfractionated or enriched in progenitor subpopulations) in numerous clinical trials worldwide. Critically reviewing the accumulated data in their totality,^{52–54} a number of conclusions can be drawn:

- (i) An excellent feasibility and safety profile has been established for intracoronary delivery of bone marrow-derived cells.
- (ii) Overall clinical outcomes have been generally positive, although primary endpoints have not always been met and sustained functional benefits remain in doubt.
- (iii) The patient population was not very ill at baseline, most having suffered their first MI with prompt reperfusion and a median ejection fraction (EF) of ~50% pre-therapy, leaving little room for improvement.

The legacy of these studies has left the field with cautious optimism, while motivating a search for better cell types. It is plausible (but still conjectural) that a stem cell source with a higher propensity to regenerate myocardium, directly and indirectly, might increase the benefits to patients.

Finally, bone marrow-derived cells have also been used for the treatment of refractory angina^{55,56} and chronic heart failure,^{57–59} albeit on a much smaller scale compared with acute MI. Early, small clinical studies have shown some hints of efficacy; however, primary efficacy

endpoints have not been met in these underpowered studies and results have been inconsistent.

Heart-derived cells

The mammalian heart traditionally has been viewed as a terminally-differentiated organ; cardiomyocytes were believed to be subject to decades of use and potential injury with no hope of reprieve. Nowadays, the concept of endogenous mammalian heart regeneration has been firmly established; through use of radiocarbon dating of human postmortem cardiac tissue, it has been documented that cardiomyocyte turnover in the adult human heart occurs at a rate of ~1% per year, with ~40% of the mature heart composed of postnatally generated myocytes.⁶⁰ In addition, multiple populations of putative endogenous CSCs have now been identified. CSCs presumably function physiologically to offset a low, but finite basal rate of cardiomyocyte loss. (It should be noted, however, that an obligatory role for CSCs in cardiomyocyte renewal has yet to be demonstrated; an alternative path to regeneration is via re-activation of the cell cycle in adult cardiomyocytes, with or without partial dedifferentiation.^{61,62}) The number of CSCs is low (one estimate posits 1 CSC per ~10 000 cardiomyocytes),¹⁸ helping to rationalize why endogenous repair does not suffice to reverse major injury. However, because CSCs are resident in the heart and pre-programmed to reconstitute all cardiac lineages (but not extracardiac tissues), they represent a logical cell candidate to regenerate the heart iatrogenically.

Historically, the term ‘cardiac stem cells’ was first used by Deisher in 1999 to describe multipotent small, round, slowly replicating, non-adherent cells isolated from the hearts of adult P53-deficient mice.⁶³ In 2003–2004, several studies advanced the notion that the adult heart contains its own reservoir of antigenically-distinctive stem cells. CSCs, defined by an ability to differentiate into multiple cardiac lineages *in vitro* and *in vivo*, were identified in rodents by stem cell-related markers and other phenotypic properties, including c-Kit (CD117, the receptor for stem cell factor),¹⁸ Sca-1 (stem cell antigen-1)⁶⁴ and sphere-forming ability (the ability to self-organize into three-dimensional microtissues of CSCs and supporting cells).⁶⁵ The investigators showed that such cell products, when injected into the heart in post-MI models, produced multilineage differentiation (cardiomyocytes, endothelial cells, vascular smooth muscle cells) and, in some studies^{18,65} functional benefits.

Our own attention to the regenerative potential of the human heart and its possible therapeutic application was focused by the work of

Messina *et al.*⁶⁵, who first reported the isolation of CSCs from human myocardium. Working with large human cardiac surgical specimens as the source tissue, those investigators described a technically-straightforward approach to generate CSCs and supporting cells. Biopsies minced (Fig. 1A) and placed in primary culture were found, spontaneously, to shed cells (Fig. 1B and C), which could be harvested by gentle enzymatic digestion. When placed in suspension culture, these cells self-organized into spherical clusters termed ‘cardiospheres’ (CSps) (Fig. 1D), by analogy to neurospheres formed by neural stem cells. This study also showed that CSps provide an environment favoring upregulation of stemness in the proliferative core of the sphere as well as increased angiogenesis and cardiogenesis. Human CSps, when injected into post-MI SCID mouse hearts, engrafted, exhibited cardiogenic and vasculogenic differentiation, and improved heart function. This exciting work was limited by the requirement for open surgical biopsies, and by the fact that CSps would appear, from first principles,⁶⁶ to be too large (50–200 μm) to be safely delivered via the clinically-routine intracoronary route.

The Marbán laboratory adapted and miniaturized the CSp culture method to enable the use of minimally-invasive percutaneous endomyocardial biopsies as the source tissue.¹⁷ CSps were re-plated and further expanded in monolayer culture (Fig. 1E) to yield therapeutically relevant numbers (tens of millions) of cardiosphere-derived cells (CDCs) in a timely manner (4–6 weeks), despite the small amount of

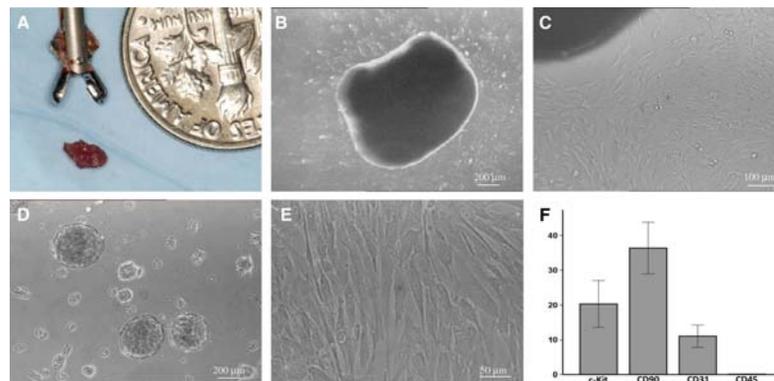


Fig. 1 Specimen processing for human cardiosphere growth and CDC expansion. Cardiac biopsies (A) are minced into fragments termed explants. Explants are placed in primary culture and spontaneously shed outgrowth cells (B) which upon confluency (C) can be harvested by gentle enzymatic digestion. When placed in suspension culture, these cells self-organized into multicellular spherical clusters, termed cardiospheres (D). CSps are collected and plated onto fibronectin-coated dishes, generating CDCs (E). Flow cytometry experiments demonstrate that CDCs are a naturally heterogeneous population of non-hematologic origin (CD45⁻), comprising endogenous cardiac stem cells (c-Kit⁺) and cardiac mesenchymal stem cells (CD90⁺).

starting tissue material. Figure 1 depicts the key steps in the method. CDCs (in contrast to antigenically-purified cells) are a naturally heterogeneous cardiac-derived cell population rich in CSCs and cardiac mesenchymal cells (Fig. 1F). When grown according to established methods, CDCs are clonogenic and exhibit multilineage potential,⁶⁷ thus fulfilling key criteria for stem cells. Moreover, CDCs can be safely delivered via the intracoronary route within a defined dosage range.⁶⁸

Over the past 6 years, we have demonstrated that CDCs can engraft, differentiate and improve cardiac function post-MI in mice,^{17,69,70} rats^{71–73} and pigs^{68,74}. Figure 2 provides a synopsis of the functional benefit produced by CDC therapy in various animal models. With regard to safety it should be noted that no tumors have been detected in >1000 experiments, no increases in toxicology signals or in arrhythmias have been observed, nor has there been excess mortality or morbidity in cell-treated groups relative to placebo controls. Moreover, at least eight independent laboratories worldwide have reproduced the published methodology and verified CDCs' identity and utility.^{75–82} On the other hand, critiques of the CSp methodology have appeared,^{83,84} but, as we have pointed out in detailed rebuttals^{67,85}, these studies did not follow published protocols for CDC isolation and expansion, and the methodological variations likely explain the negative results. The question of whether CDCs outperform other cell types is under active investigation. In small comparative studies, CDCs outperformed MSCs *in vitro*⁸¹ and *in vivo*.⁷⁸ In a head-to-head comparison of four different cell types (CDCs, BMMNCs, bone marrow-

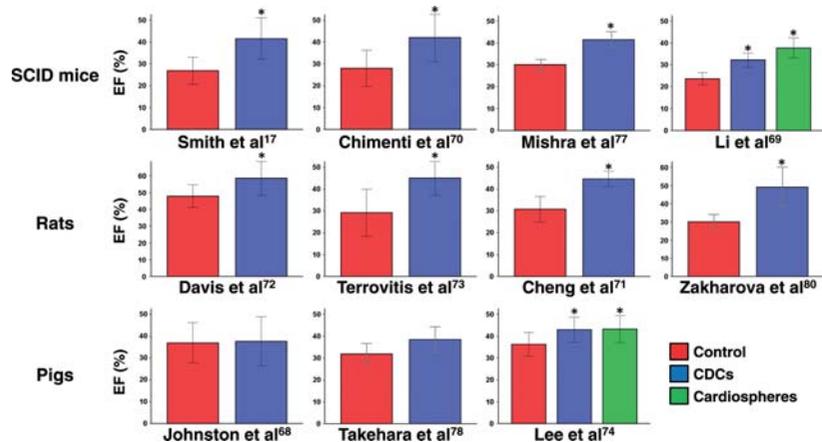


Fig. 2 CDCs and CSps improve cardiac function post-MI in mice, rats and pigs, compared with sham-injected controls (* $P < 0.05$ compared with controls; in the study by Zakharova *et al.*, CDCs were transplanted as a sheet, while in the study by Takehara *et al.*, CDCs were injected with a basic fibroblast growth factor incorporating hydrogel).

derived MSCs and adipose-derived MSCs) in the same animal model in the same laboratory, CDCs emerged as superior in terms of paracrine factor secretion, angiogenesis, cardiomyogenic differentiation, ischemic tissue preservation, anti-remodeling effects and functional benefit post-MI.⁸⁶ Interestingly, the natural mixture of CDCs also outperformed purified c-Kit+ CSCs.⁸⁷ This finding suggests that the non-CSC subpopulation supports the survival and engraftment of the CSC subpopulation, translating into greater efficacy of the mixture relative to the purified fractions. However, it is equally possible that the c-Kit+ subpopulation is irrelevant to the mechanism of benefit.

With regard to clinical translation, a phase I/II clinical study of CDCs—the CADUCEUS (CARDiosphere-Derived aUtologous stem CELLS to reverse ventricUlar dySfunction) study, is under way;⁸⁸ human subjects with recent acute MI (2–3 months post-MI) and left ventricular dysfunction (EF, 25–45%) receive intracoronary infusion of 12.5–25 million autologous CDCs harvested from endomyocardial biopsies, or conventional treatment, by prospective randomized assignment. Enrollment is complete, and full 6-month follow-up data will be available by the end of 2011. Another trial (NCT00474461)⁸⁹ of heart-derived cells is ongoing in post-CABG patients with heart failure; a total of 0.5–1 million c-Kit-purified heart-derived cells are infused intracoronarily 4 months after tissue harvesting during surgery. The natural tendency of function to improve slowly and progressively after CABG^{90,91} may complicate the interpretation of this trial.

Areas of agreement

A number of generalizations (summarized in Table 1) arise from the BMMNC acute MI studies, which by far are the most numerous to date: the safety profile has been quite favorable; efficacy, as gauged by an increase in EF, has been inconsistent and, overall, modest, while other clinical endpoints are more favorably affected; retention and engraftment of cells are disappointingly low and, as a corollary, the beneficial effects of cell therapy seem to be related to paracrine effects,

Table 1 Areas of agreement and controversy after the first decade of clinical application of bone marrow-derived cells for heart repair.

Areas of agreement	Areas of controversy
Excellent safety profile of IC delivery	Cardiomyogenic potential of bone marrow cells
Modest, inconsistent efficacy	Clinical relevance of observed benefit
Poor acute retention and long-term engraftment	'Numbers paradox'
'Paracrine hypothesis'	

rather than direct new tissue formation. These points are considered individually below.

Safety of intracoronary delivery

So far, no safety concerns regarding delivery of BMMNCs through a recently reopened infarct-related coronary artery have emerged. Figure 3 depicts the excellent profile of intracoronary infusion of BMMNCs, with respect to several clinical safety endpoints. Infusion of cells does not inflict additional ischemic damage to the myocardium.¹⁴ No increased incidence of arrhythmias or increased arrhythmia inducibility¹⁴ has been reported. Importantly, in-stent/culprit artery restenosis (considered as a potential risk due to cell-mediated plaque angiogenesis or plaque inflammation based on experimental studies in mice⁹²) was increased in only one small, non-randomized study⁹³; however, recent meta-analyses^{53,54} show no excess proclivity to target-vessel restenosis or repeat revascularization in patients treated with BMMNCs. Finally, there is no evidence of increased tumorigenesis after BMMNC therapy.

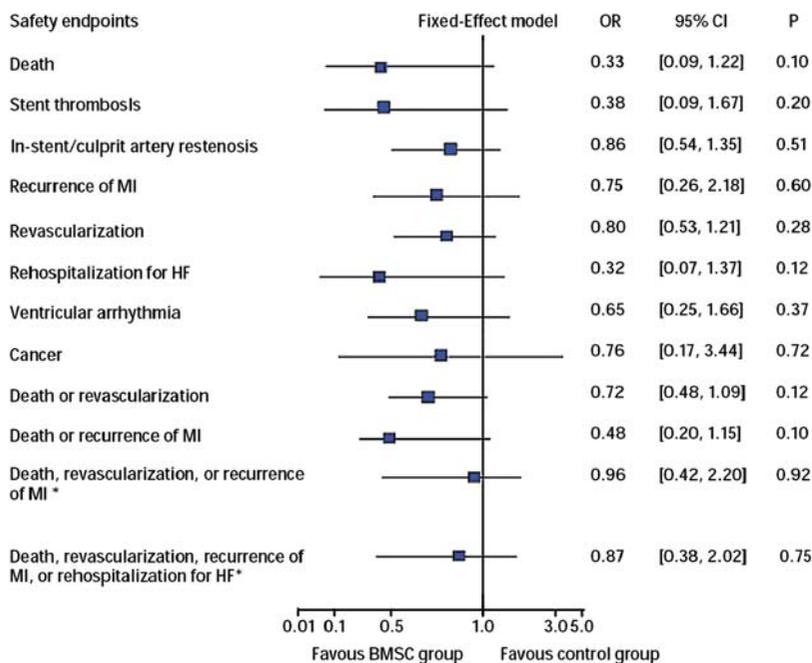


Fig. 3 Excellent safety profile of intracoronary delivery of BMMNCs. Odds ratio of BMMNC infusion therapy with respect to several safety endpoints. Five trials (REPAIR-AMI,^{13,96} BOOST,¹⁴ ASTAMI,¹⁵ Janssens *et al.*⁹¹ and Yao *et al.*¹⁴⁴) were included in the meta-analysis. Reproduced from ref. 53.

Overall, the safety profile of intracoronary delivery of BMMNCs has been excellent.

Hints of efficacy

As with SKMs, early small and uncontrolled pilot studies of BMMNC delivery after acute MI demonstrated consistently positive results.^{94,95} However, later large randomized studies have yielded varied results. REPAIR-AMI showed a significant improvement in EF at 4 months,⁹⁶ while the similarly-designed ASTAMI showed no functional benefit,¹⁵ a difference which has since been attributed to the quality of the cells as measured by an *in vitro* cell migration assay.⁹⁷ A separate randomized but not placebo-controlled study (BOOST) concluded that the functional benefit seen at 6 months¹⁴ was not sustained at 18 months.⁹⁸ In other large randomized studies, functional benefits of BMMNC therapy were marginally positive (FINCELL,⁹⁹ REGENT¹⁰⁰), mixed [i.e. no difference in EF but decrease in scar size (Janssens *et al.*¹⁰¹) or increase in myocardial viability (BONAMI¹⁰²)] or completely negative (HEBE,¹⁰³ SCAMI¹⁰⁴). A recent meta-analysis (including 811 patients participating in 13 randomized trials) showed modest but significant benefit following BMMNC therapy: LVEF increased by ~3%, end-systolic volume decreased by ~5 ml and scar size decreased by ~3.5% in the cell-treated groups compared with controls.⁵⁴ Subgroup analysis revealed that the benefit of cell therapy was greater when cells were infused within 7 days following infarction (the optimal time window seems to be 5–7 post-MI^{52,54}) and when the dose administered was >100 million BMMNCs.⁵⁴ Despite the inconsistency of the improvements in EF, long-term clinical outcomes are reportedly improved after BMMNC therapy, as discussed below.

Low cell retention and engraftment

Low cell retention and engraftment after cell delivery to the heart are persistent obstacles to successful myocardial regeneration. Numerous studies have investigated short-term cell retention and long-term engraftment both in experimental animals^{105,106} and in humans^{107,108} and the results have been overwhelmingly disappointing; acute cell retention (i.e., within 24 h of delivery) in the heart is generally <10%, regardless of the cell type or delivery route. Cells acutely lost from the heart are often washed out via the coronary venous system or mechanically ejected via the injection site, while retention rates in beating hearts are markedly lower than in non-beating hearts.⁷³ Importantly,

~90% of the successfully-retained cells die within the first week¹⁰⁹ most probably due to ischemia, inflammation or anoikis (apoptosis due to detachment from the extra-cellular matrix), and <1% of transplanted cells can be identified 4 weeks after transplantation.¹¹⁰ Taking into account that studies of MSCs¹¹¹ and CDCs^{71,73} reveal a strong correlation between engraftment rate, even if low, and long-term functional benefit, there is good reason to believe that the development of more effective delivery methods, combined with successful means of boosting transplanted cell retention and engraftment, would significantly enhance the utility of cell therapy.

Paracrine hypothesis

In the vast majority of experimental studies, the number of differentiated myocytes derived from transplanted stem cells is too small to account for the observed improvements in cardiac function.¹¹² This can be attributed to the combination of low long-term cardiac engraftment and the low cardiomyogenic potential of the majority of adult stem cell types used for heart regeneration. Thus, the prevailing concept of adult stem cell efficacy has shifted towards the 'paracrine hypothesis', according to which the transplanted cells are proposed to produce soluble factors that are beneficial to the infarcted heart.¹¹³ Indeed, SKMs,¹¹⁴ bone-marrow-derived cells¹¹⁵ and cardiac-derived cells⁷⁰ produce and secrete a broad variety of cytokines, chemokines and growth factors that are known to be involved in cardiac repair, and hypoxic stress increases the production of several of these factors. In addition, strong support of a paracrine mechanism for cardiac repair comes from experimental studies in which the administration of conditioned medium is able to recapitulate, at least partially, the beneficial effects observed after stem cell therapy.¹¹⁶ Potential effects of paracrine factors include cytoprotection of resident myocytes, upregulation of angiogenesis, modulation of inflammatory processes resulting in better infarct healing, improved cardiac metabolism and contractility, promotion of cardiomyocyte cell cycle re-entry, recruitment of endogenous stem cells, and induction of secondary humoral effects in the host tissue.^{113,117} It is encouraging that functional benefit can be achieved through indirect pathways; this mechanism of action rationalizes the persistence of benefit despite the evanescence of transplanted cell survival, in that the new tissue originates from the recipient heart rather than from the transplant.

Areas of controversy

Cardiogenic potential of bone marrow-derived cells

The question of whether bone marrow-derived cells in general, and hematopoietic cells in particular, can transdifferentiate into cardiomyocytes has been hotly debated for a decade. The controversy began with a report showing extensive engraftment and cardiogenic differentiation of bone marrow-derived hematopoietic cells after injection in infarcted hearts of mice.⁴³ In addition, studies examining postmortem human cardiac tissue from male patients who had received hearts from female donors reported cardiac cellular chimerism; Y-chromosome positive cardiomyocytes (indicating extracardiac origin) were detected in the transplanted female hearts, but the extent of the phenomenon varied greatly (from 0.04 to 10%, a difference of ~250 fold) among studies.^{118,119} However, several groups have since contested the claims that bone marrow cells can readily acquire a cardiac phenotype,^{44,45} while subsequent studies have argued that what originally was interpreted as transdifferentiation may have arisen from cell fusion.¹²⁰ Currently, no consensus exists on whether bone-marrow derived progenitor cells can meaningfully differentiate into cardiomyocytes *in vivo*,⁴⁷ although skepticism prevails.

Is the observed effect of cell therapy on global function meaningful from a clinical standpoint?

Taking into account the small effect of BMMNC therapy on EF, it is intriguing that significant benefits on clinical endpoints have been reported. In the REPAIR-AMI trial, the incidence of the pre-specified cumulative endpoint of death, MI or necessity for revascularization was significantly lower 1 year after cell therapy, even though the study was not powered to detect differences in clinical endpoints.¹³ Likewise, the combined endpoint of death, recurrence of MI, and rehospitalization for heart failure was reduced in patients receiving intracoronary BMMNC administration.¹³ These favorable clinical outcomes were sustained at 2 years of follow-up.¹²¹ In another study (BALANCE), an early significant improvement in EF and infarct size at 3 months and 1 year was followed at 5 years by greater exercise capacity and lower mortality in the treated patients.¹²² Finally, trends in favor of BMMNC therapy with regard to hard clinical endpoints have also emerged from meta-analyses.^{53,54}

So, how can one reconcile equivocal functional benefit with extraordinary clinical outcomes? Relevant considerations include the

following: (i) EF (which is load dependent) may not be the best-suited index for assessing the effects of cell therapy, given the hearts' inherent ability to compensate for loss of contractility by increases in preload. To that extent, other metrics (such as attenuation of ventricular remodeling,¹²³ enhanced end-systolic elastance, or decrease in scar size) may be more appropriate. (ii) The patient population in these trials was not very ill at baseline (median EF ~ 50%); most patients suffered their first MI and received prompt reperfusion and state-of-the-art medical therapy, leaving little room for additional improvement. However, a substantial functional benefit is detected in the patients with larger myocardial infarcts (increase in EF of ~7.5% in the REPAIR-AMI trial), indicating that specific patient populations (those with the worst prognosis) may benefit preferentially from cell therapy¹³ (more on that later). (3) The effects of BMMNC therapy are comparable to what is achieved by established therapeutic strategies including primary PCI, thrombolysis, angiotensin-converting enzyme inhibition or β -blocker therapy, which are used routinely in clinical practice and confer a survival benefit.¹²⁴ Nevertheless, well-powered large-scale clinical trials focusing on hard clinically-meaningful endpoints are mandatory to determine whether the observed functional improvement indeed translates into increased survival and reduced morbidity.

The 'numbers paradox'

A major challenge in regenerative medicine is the sheer number of cells that need to be replaced; in a typical MI, ~1 billion cardiomyocytes are lost.¹²⁵ As a result, when reviewing data from clinical studies, we are faced with a 'numbers paradox': cell transplantation translates into a range of beneficial effects, but these effects occur in a setting characterized by poor cell retention and minimal long-term survival as well as negligible cardiomyogenic transdifferentiation. For the sake of argument, let us consider the study by Janssens *et al.*¹⁰¹; there, intracoronary infusion of ~170 million BMMNCs was associated with a reduction in infarct size at 4 months (measured by cardiac MRI) compared with placebo infusion; cell therapy induced an additional decrease in scar mass of ~3 g (equivalent to ~60 million cardiomyocytes, since 1 g of human myocardium contains ~20 million myocytes). Taking into account that acute retention of bone marrow-derived cells after intracoronary infusion in humans is 1–2%¹⁰⁸ (measured by administration of ¹⁸F-FDG-labeled cells and PET), the 'numbers paradox' becomes clear: how can 2–4 million acutely retained non-myogenic cells (~90% of which will most probably die within the first week¹⁰⁹ without compensatory proliferation, resulting

in miniscule long-term engraftment¹¹⁰) lead to the production of 60 million new cardiomyocytes 4 months after cell administration? Potential mechanisms include cardioprotection of resident myocytes, recruitment of endogenous regeneration or cardiomyocyte cell cycle re-entry, upregulation of angiogenesis leading to better infarct healing, scar contraction without myogenesis and hypertrophy of resident myocytes at the infarct border zone.^{113,126} Nevertheless, clinical efficacy should not be dismissed just because mechanisms are not understood. The history of therapeutics is littered with well-reasoned failures and adorned by serendipitous triumphs.

Future directions

The results of the first generation of clinical trials have been mixed; SKMs have been resoundingly negative, and the BMMNC experience, while a lot more positive, leaves much to be desired. The pursuit of improved methods for cell delivery, means to boost retention and engraftment, more potent and better-standardized stem cell products, and more apt patient populations is certainly merited.

Delivery routes

So far, stem cells for heart repair have been delivered clinically via three routes: systemic intravenous infusion, intracoronary infusion, and intramyocardial injection (either by direct open-chest injections as an adjunct to CABG or by transendocardial catheter-based injections),¹²⁷ while retrograde coronary venous,¹⁰⁵ transvenous intramyocardial^{128,129} and intrapericardial approaches¹³⁰ have been used mostly under experimental settings. Intravenous infusion, albeit simple in execution, is hampered by trapping of cells in the lungs; therefore, only a small number of cells reach the coronary circulation and are available for transendothelial migration into the myocardium.¹³¹ Intracoronary delivery of cells into a recanalized infarct-related artery is safe and convenient (can be performed with standard balloon catheters) and has the inherent advantage that cells are infused into myocardial regions with preserved oxygen and nutrient supply, thus ensuring a favorable environment for cell survival. However, retention of cells is suboptimal (most cells are washed away before they can migrate into the surrounding tissue), and unperfused regions of the myocardium are inaccessible. In addition, safety concerns are raised during infusion of larger cells due to potential capillary plugging and microinfarction; this problem, if recognized, can be overcome by appropriate cell dosing and

optimization of the infusate⁶⁸ (it should be noted, however, that in the vast majority of pre-clinical and clinical studies to date, dosing has, by and large, been non-systematic, guided more by feasibility and accessibility than by intentional dosage optimization; a remarkable 6700-fold range in cell dose is observed in human bone marrow trials¹³²). All things considered, the intracoronary delivery route seems to be best-suited for the treatment of acute MI. On the other hand, direct intramyocardial cell injections result in better retention of cells compared with intracoronary or systemic approaches,¹³¹ which may account for the superior functional benefit associated with this route of administration.⁷⁴ Since freshly infarcted myocardium might run a higher risk of perforation (a reasonable but unverified conjecture), intramyocardial cell injections are probably more suitable for patients with chronic ischemic cardiomyopathy. With regard to the latter, we have shown that, in a mini-pig model of ischemic cardiomyopathy, direct open-chest injection of cardiac-derived cells provides greater functional benefit than intracoronary delivery.⁷⁴ Since open-chest injections would be unlikely to gain clinical acceptance unless adjunctive to clinically indicated surgery, less-invasive catheter-mediated methods of intramyocardial delivery (preferably employing electromechanical mapping and identification of viable myocardium¹³³) need to be explored.

Finally, when it comes to cardiac-derived cell products, another advantage of intramyocardial injections is the potential for safe administration of CSps for heart repair. CSps, due to their larger size of 50–200 μm , are expected to embolize at the arteriolar level and thus may not be safe to administer intracoronarily. We have found that intramyocardial delivery of CSps disproportionately boosts cardiac function in small⁶⁹ and large animal⁷⁴ models of ischemic cardiomyopathy compared with monolayer-cultured CDCs. This effect can be attributed to the three-dimensional multilayer composition of CSps, yielding a three-dimensional microtissue product with an upregulation in ‘stemness’ and increased expression of adhesion molecules, enabling improved cardiomyogenesis and more robust engraftment.⁶⁹ These findings provide good reason to favor CSps in future direct injection studies.

Strategies to boost retention and increase engraftment

A major challenge to the effectiveness of cell therapy is the low percentage of cell retention after delivery into the heart, whether by direct intramyocardial injection or by intracoronary infusion. Multiple lines of evidence indicate that strategies that effectively boost acute retention translate into greater functional benefit downstream.^{71,73} Several

different methods have been used including priming of host tissue to increase homing, preconditioning of transplanted cells with cytokines, prosurvival factors and physical stimuli, genetic engineering of cells and the use of biomaterial scaffolds.^{134,135} While much work has focused on improving transplanted cell quality and creating a more hospitable host environment, most cells are washed away too quickly to be effective. Physical approaches to retain cells in the diseased tissue long enough to enable biological integration may boost treatment efficacy, and would be synergistic with efforts to improve cell quality or environmental receptiveness. Strategies to enhance acute retention, if successful, stand to boost the long-term efficacy of cell therapy. This claim is supported by the results of two interventions which we have implemented in rat models of direct intramyocardial injection: capping the injection site with fibrin glue to prevent back-flux of injected cells,⁷³ and using magnetic targeting to retain iron-loaded CDCs within the heart.⁷¹

Use of allogeneic cells

During the first decade of cell therapy for human heart regeneration, the vast majority of clinical trials have been conducted using autologous cells. Autologous sources are attractive because immunologic rejection is avoided by default. Nevertheless, autologous therapy is associated with serious limitations, which complicate widespread clinical application. Specifically, autologous therapy necessitates patient-specific tissue harvesting, cell processing and quality control, which pose significant logistic, economic and timing constraints. In addition, stem cell growth properties and plasticity may be hampered by age and comorbidities,¹³⁶ resulting in interpatient variability in cell potency. The use of allogeneic cells, if safe and effective, would obviate such limitations, enabling the generation of highly standardized 'off the shelf' cellular products. The obvious disadvantage of allogeneic therapy is the risk of immune rejection. To that end, MSCs, which are purportedly immunoprivileged, have attracted interest; clinical trials involving the administration of proprietary allogeneic human MSCs to patients with heart disease are already under way, and the preliminary results have been encouraging.^{137,138} It should be noted that, without immunosuppression or HLA matching, most allogeneic cells (even MSCs) will eventually be rejected after *in vivo* transplantation.¹³⁹⁻¹⁴² Nevertheless, since the vast majority of the observed functional benefit is attributable to indirect pathways, rejection of allogeneic cells may not be an issue if it is delayed long enough to allow them to exert their protective and regenerative paracrine effects.

Targeting a sicker patient population

The main patient population used in the first generation of clinical trials has been a first-infarct population, with little ventricular dysfunction, and a projected low mortality and morbidity even without adjunctive biological therapy. However, data from various different trials suggest that patients with more severe MI (the ones with the worse prognosis) benefit most from cell therapy. In the REPAIR-AMI study, patients with a lower baseline EF (<48.9%) showed a significant, 3-fold higher recovery in global EF than seen in the converse group. In addition, the beneficial effect on clinical endpoints was also preferentially observed in those patients with a lower baseline EF after myocardial infarction.¹³ In the REGENT study, significant functional benefit was observed only in cell-treated patients who had baseline LVEF <37%.¹⁰⁰ In BOOST, only patients with larger infarcts and greater infarct transmuralities demonstrated sustained functional improvement at later time points,⁹⁸ while in the study by Janssens *et al.*¹⁰¹ cell therapy led to enhanced recovery of regional function only in the most severely infarcted myocardial segments (characterized by the greatest infarct transmuralities). Finally, in the TOPCARE-CHD registry, NT-proBNP serum levels >735 pg/ml at baseline were an independent predictor of a favorable response 3 months after intracoronary administration of BMMNCs.¹⁴³ Taken together, the results indicate that the greatest benefits of stem cell therapy occur in patients with the greatest infarct-induced myocardial damage. This finding has major implications for the design of future clinical studies: cell therapy can maximize its potential for successful myocardial repair and regeneration by targeting a sicker patient population.

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