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Haematopoietic stem cells and repair of the ischaemic heart

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HSCs (haematopoietic stem cells) are multipotent stem cells that give rise to all cells of the blood cell lineage. In recent years, it has been proposed that bone marrow serves as a reservoir for cardiomyogenic precursors and that, following cardiac injury, these stem cells circulate to the site of injury where they contribute to myocardial repair and regeneration. This concept of stem cell plasticity has been controversial and, in fact, several key studies on the cardiomyogenic potential of HSCs have not been reproducible in the hands of independent investigators. Despite this controversy, the clinical community has pushed forward with clinical trials of bone marrow transplantation for the treatment of ischaemic heart disease. The following review summarizes the mechanistic underpinnings of bone marrow transplantation into ischaemic myocardium, focusing on the basic science that forms the foundation of this field, and highlights the controversies and new avenues for research that have emerged. It also describes the current state of the art in clinical trials of bone marrow transplantation for heart failure.

INTRODUCTION

Congestive heart failure is a leading cause of death in industrialized nations. In the U.S. alone, over 500 000 new cases are diagnosed yearly [1]. The mainstay of treatment is lifestyle modification and pharmacotherapy, including treatment with β-blockers and ACE inhibitors (angiotensin-converting enzyme inhibitors), but even with optimal medical therapy, 1-year mortality is nearly 20 %. Because other treatment options, including heart transplantation, ventricular assist devices and the use of artificial hearts, are not readily available to most patients, there has been a tremendous need for the development of novel treatment strategies for heart failure. In recent years, one emerging therapy has been cell-based myocardial repair.

MYOCARDIAL REGENERATION

Historically, the adult heart has been viewed as a terminally differentiated organ without the capacity for self-renewal or regeneration. Recent data challenge this doctrine, suggesting that innate mechanisms for myocardial regeneration exist (Figure 1). Several key studies are reviewed below and summarized in Figure 2.

In 1998, Kajstura et al. [2] presented data that low-level myocyte proliferation occurs in normal hearts and that this process is augmented nearly 10-fold in end-stage IHD (ischaemic heart disease) and idiopathic dilated cardiomyopathy. Using confocal microscopy, they found that 14 myocytes/million were in mitosis in control human hearts, whereas 152 myocytes/million and 131 myocytes/million were in mitosis in hearts from
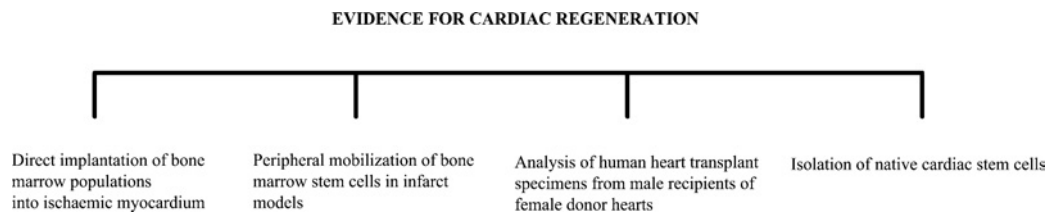
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1. STEM CELL PLASTICITY

Bone marrow stem cell   +   injured cardiac local environment   →   cardiomyocyte transdifferentiation

2. RESIDENT CARDIAC STEM CELL DIFFERENTIATION

Resident cardiac stem cell   +   injured cardiac local environment   →   cardiomyocyte differentiation

Figure 1   Proposed mechanisms for cardiac regeneration

The schematic diagram summarizes two possible mechanisms for cardiac regeneration in the adult. In the 'stem cell plasticity' mechanism, injury in the cardiac microenvironment results in mobilization of bone-marrow-derived stem cells (which may include HSCs or MSCs), which then home to the injured heart and transdifferentiate into cardiomyocytes. In the 'resident cardiac stem cell differentiation' mechanism, injury in the cardiac microenvironment provides a cue to native cardiac stem cells, which then proliferate and differentiate into cardiomyocytes.

EVIDENCE FOR CARDIAC REGENERATION

Direct implantation of bone marrow populations into ischaemic myocardium

Peripheral mobilization of bone marrow stem cells in infarct models

Analysis of human heart transplant specimens from male recipients of female donor hearts

Isolation of native cardiac stem cells

Figure 2   Schematic diagram illustrating the principal areas of research from which the concept of cardiac regeneration is derived

patients with end-stage IHD and idiopathic dilated cardiomyopathy respectively. This study provided evidence that low-level myocyte proliferation occurs in the adult heart and that injury is a stimulus for increased proliferation.

A follow-up study by Beltrami et al. [3] in 2001 also found evidence for myocyte proliferation after myocardial infarction in humans. Samples from the border-zone and distant myocardium were examined in 13 patients who died 4–12 days after infarction. The fraction of myocytes undergoing mitosis was 0.08 % in the border-zone and 0.03 % in the distant myocardium. This study provided further evidence that the adult heart is capable of intrinsic regeneration and repair after injury.

In 2002, Quaini et al. [4] published data that cardiac regeneration occurs following orthotopic heart transplantation in humans. In a series of eight male patients receiving female donor hearts, they found recipient-derived cardiomyocytes and vascular structures within the donor hearts at autopsy. The Y chromosome was used as a marker of recipient cells using the technique of fluorescence *in situ* hybridization. Amazingly, this group found that $9 \pm 4\%$ of the myocytes, $10 \pm 3\%$ of the arterioles and $7 \pm 1\%$ of the capillaries contained the Y chromosome. Moreover, cardiac regeneration was noted as early as 4 days after transplantation. Several other groups have also found cardiomyocytes of recipient origin in transplanted human hearts, although most report much lower numbers. Laflamme et al. [5] reported a mean of 0.04 % recipient-derived cardiomyocytes in autopsy specimens of five males receiving female donor hearts. Hocht-Zeisberg et al. [6] also reported a mean of 0.04 % recipient-derived cardiomyocytes in a series

of 14 heart transplant patients. Muller et al. [7] found $0.16 \pm 0.04\%$ recipient-derived cardiomyocytes in right ventricular biopsy specimens from 13 male patients who underwent gender-mismatched heart transplantation.

Which cells contribute to myocardial regeneration?

With early studies supporting the notion that adult cardiac regeneration occurs at low levels, the following question emerged: which cells contribute to myocardial regeneration? Do resident cardiac stem cells exist or do non-cardiac stem cells contribute to myocardial regeneration?

In recent years, the topic of stem cells has been of enormous scientific and political interest. Stem cells are cells that are capable of self-renewal which can differentiate into one or more lineages. Totipotent stem cells are capable of forming a fully functional organism. Pluripotent stem cells can differentiate into nearly all cells arising from the three germ layers. Multipotent stem cells can differentiate into a limited range of differentiated lineages that are appropriate to their tissue location.

Stem cells can be characterized based on their cell-surface markers, functions and the proteins they express. Stem cells exist in both the embryo and the adult, and these are called embryonic stem cells and somatic stem cells respectively. Experimental studies *in vitro* have shown that embryonic stem cells can be induced to differentiate into beating cardiomycytes [8,9], and that these cells can then be implanted into adult myocardium after infarction to effect myocardial regeneration and repair [10]. However, ethical issues restrict the availability of

embryonic stem cells for myocardial restoration, and research has focused on somatic stem cells.

Plasticity of the adult bone-marrow-stem cell

An extraordinary series of experiments emerged in the early part of this decade supporting the notion that adult bone marrow stem cells may have an unexpected degree of plasticity. That is, when taken outside their normal tissue environment, bone-marrow stem cells may have the capacity to transdifferentiate into other cell types. Stem cells in the bone marrow include MSCs (mesenchymal stem cells) and HSCs (haematopoietic stem cells). MSCs are self-renewing clonal precursors of non-haematopoietic stromal tissues; they can be isolated from the bone marrow and expanded in culture based on their ability to adhere to culture dishes and proliferate *in vitro*. HSCs give rise to all haematopoietic lineages and have been isolated based on cell-surface markers. In murine models, HSCs are c-kit$^+$, Thy-1$^+$, Lin$^-$ (lineage-negative) and Sca-1$^+$ cells, and in humans, HSCs are CD34$^+$ and CD133$^+$.

In recent years, reports of MSC transdifferentiation into osteogenic, chondrogenic, adipogenic [11] and cardiomyogenic [12] lineages have been published. Xu et al. [13] demonstrated that treatment of MSC with 5-azacytidine can result in differentiation into a cardiomyocyte phenotype *in vitro*. Moreover, Toma et al. [12] found that 2 weeks after implantation of lacZ-labelled human MSCs into the uninjured left ventricle of immunodeficient mice, rare β-gal$^+$ (β-galactosidase-positive) cells expressing myogenic proteins desmin and troponin T could be identified.

Numerous studies have suggested that unfractionated bone marrow cells or bone marrow cells enriched by various methods for HSCs could contribute to multiple non-haematopoietic tissues as well, including neurons [14], hepatocytes [15], skeletal muscle [16,17] and cardiomyocytes [18]. Jackson et al. [18] lethally irradiated mice and then reconstituted their bone marrow with lacZ-labelled HSCs. At 10 weeks later, cardiac injury was induced by occluding the LAD (left anterior descending) coronary artery for 60 min. After 2–4 weeks, they found 0.02 % of cardiomyocytes and 3.3 % of endothelial cells in the peri-infarct region were β-gal$^+$. This study lent support for the notion that bone-marrow-derived HSCs can regenerate myocardium. In 2001, Orlic et al. [19] took this study one step further; rather than rely on innate bone-marrow-derived mechanisms for repair after myocardial injury, they directly transplanted 1×10^5 Lin$^-$c-kit$^+$ (HSC-enriched) bone marrow cells into the border-zone myocardium of mice several hours after LAD ligation. Amazingly, they found new donor-cell-derived myocardium comprising 68 % of the infarct 9 days after transplantation. New donor-derived myo-

cytes, endothelial cells and smooth muscle cells were detected. Moreover, ventricular function and haemodynamics were significantly improved in cell-treated animals compared with negative controls. This study drew enormous attention from both the scientific and clinical communities and set the stage for early human trials of bone marrow transplantation into the infarcted heart.

More evidence for a bone-marrow-derived source for myocardial regeneration: lessons from human transplant models

As experimental models emerged supporting the hypothesis that bone-marrow-derived cells can transdifferentiate into cardiomyocytes, a clinical study by Deb et al. [20] in human bone marrow transplantation provided further evidence. They found that, in the hearts of female recipients of gender-mismatched bone marrow transplants, 0.23 ± 0.06 % of the cardiomyocytes were Y chromosome$^+$. Thus it appeared that a small percentage of cardiomyocytes were derived from the donor bone marrow.

Mobilization of bone marrow stem cells: augmenting innate repair mechanisms

As growing data supported the theory that bone-marrow-derived cells are involved in low-level myocardial regeneration after injury, the question was asked how can we augment this natural repair system *in vivo*? One hypothesis was that peripheral mobilization of bone marrow stem cells with cytokines might augment the system. In 2001, Orlic et al. [21] reported that G-CSF (granulocyte colony-stimulating factor)- and SCF (stem cell factor)-mediated bone marrow cell mobilization in infarcted mice resulted in decreased mortality, decreased infarct size, regeneration of myocytes and vascular structures, and improved cardiac function. A similar trial in non-human primates failed to detect myocardial repair, although increased local perfusion, presumably secondary to increased angiogenesis, was noted in treated groups [22].

Confusion over cell fusion

The concept that bone marrow stem cells have plasticity to differentiate into different cell types depending on their local environment challenged a long-held dogma of developmental biology: that tissue-specific stem cells derive from the tissue that they repair. Transdifferentiation was met with a great deal of scepticism in stem cell biology circles, and several alternative theories were put forward to explain the unexpected scientific findings.

A series of experiments by Wagers et al. [23] critically examined the hypothesis that HSCs can give rise to non-haematopoietic cells *in vivo*. Using a model in which bone marrow of lethally irradiated mice was reconstituted with a single GFP$^+$ (green fluorescent protein-positive)

HSC, they found little contribution of HSCs to non-haematopoietic tissue. In their analysis of cardiac tissue in these animals, not a single donor HSC-derived cardiomyocyte was detected. In a parallel series of experiments, they used a parabiotic mouse model to look for chimaerism in non-haematopoietic tissues. GFP-transgenic/wild-type parabionts developed substantial haematopoietic chimaerism over the study period, but chimaerism was not noted in non-haematopoietic tissue.

In 2002, the concept of fusion [24] emerged as an alternative explanation for transdifferentiation events. Both Terada et al. [25] and Ying et al. [26] found that in co-culture experiments with bone marrow cells and embryonic stem cells or neural cells and embryonic stem cells respectively, tetraploid hybrids could be detected that adopt embryonic stem cell characteristics. A subsequent study by Alvarez-Dolado et al. [27] used a Cre-*lox* recombination system to detect cell fusion events in lethally irradiated mice transplanted with labelled bone marrow cells. Fusion of bone marrow cells with Purkinje cells, hepatocytes and cardiomyocytes was found. Camargo et al. [28] also used a single HSC transplant model to demonstrate that HSC-derived hepatocytes in fact occur as a result of cell fusion. Interestingly, Oh et al. [29] found that Sca1$^+$ cardiac progenitor cells delivered intravenously 6 h after cardiac ischaemia/reperfusion differentiate into myocytes within the infarct border-zone, with approximately half of these cells representing fusion products between native myocytes and the infused stem cells. In this model, they were unable to determine whether fusion preceded differentiation or vice versa.

Re-examining the data on myocardial regeneration with HSC-enriched bone marrow

In response to the 2001 study of Orlic et al. [19], which demonstrated that local implantation of HSC-enriched bone marrow into recently infarcted myocardium resulted in myocardial regeneration, several groups, including ours, attempted to reproduce and extend these unexpected findings. Orlic et al. [19] studied a single time point, 9 days after infarction, finding that when 1.5×10^5 Lin$^-$c-kit$^+$ bone marrow cells were implanted into the border-zone myocardium 6 h after infarction, fluorescent immunostaining techniques using myocardial and vascular markers identified extensive regeneration of myocardium at the 9-day post-infarction study point.

We performed a series of experiments to examine the ability of c-kit-enriched bone marrow, Lin$^-$c-kit$^+$ bone marrow, and purified HSCs to regenerate myocardium after infarction [30]. After inducing myocardial ischaemia by LAD ligation in mice, donor bone marrow cells were implanted into the border-zone myocardium. Histological analysis using confocal microscopy was performed at 10 and 30 days after infarction. We found that,

at 10 days after infarction, large number of cells were found in the areas of injection, but that by 30 days, the number of cells was much fewer. Thus 'engraftment' into the myocardium was not stable, but rather was transient. Moreover, the donor cells we identified were small and round, maintaining a haematopoietic phenotype. By co-staining with a variety of markers, we found that many of the donor cells in the c-kit-enriched donor group stained with the pan-haematopoietic marker CD45; in addition, nearly all of the donor cells in the Lin$^-$c-kit$^+$ and HSC donor groups were CD45$^+$ and stained with the myeloid (neutrophil) marker Gr-1. We concluded that, when bone marrow progenitors and HSCs are implanted into ischaemic myocardium, they do not transdifferentiate into cardiomyocytes; rather they adopt traditional haematopoietic fates, differentiating primarily into neutrophils. Using a series of functional studies, we did demonstrate, however, that implantation of stem-cell-enriched bone marrow into ischaemic myocardium protected against ventricular remodelling and resulted in preservation of left ventricular function. One hypothesis to explain this functional benefit in the absence of myocardial regeneration is that cell treatment may result in increased angiogenesis through local release of growth factors.

Nygren et al. [31] published similar results for their series of experiments studying the fate of implanted bone marrow into ischaemic myocardium in mice. Using labelled whole bone marrow and Lin$^-$c-kit$^+$ cells as donor cells, they found that cells implanted into ischaemic myocardium after LAD ligation or cryoinjury maintained haematopoietic characteristics. Using both a 9-day and 28-day study point, they found transient engraftment of CD45$^+$ cells at the sites of injection; that is, many more donor cells were noted at the 9-day study point when compared with at 28 days. Nygren et al. [31] also examined the ability of cytokine-induced stem cell mobilization to result in myocardial repair after infarction using a lacZ-transgenic mouse model in which the native bone marrow had been replaced with GFP-labelled bone marrow. At 28 days after infarction, the majority of engrafted GFP$^+$ cells within the heart were also CD45$^+$. Rare GFP$^+$ cells in the myocardium stained with cardiomyocyte markers (0.75 % of all GFP$^+$ cells analysed), but these were all lacZ$^+$ as well. This provided conclusive evidence that bone-marrow-derived cardiomyocytes were products of cell fusion rather than transdifferentiation.

Finally, Murry et al. [32] used a genetic reporter system to demonstrate that local implantation of HSC-enriched bone marrow into recently infarcted myocardium does not result in myocardial regeneration. Mice were studied 1–4 weeks after infarction and implantation of Lin$^-$c-kit$^+$ cells into the peri-infarct zone; only small round donor-derived cells were noted, and none of these co-stained with cardiomyocyte markers. Murry et al. [32] also
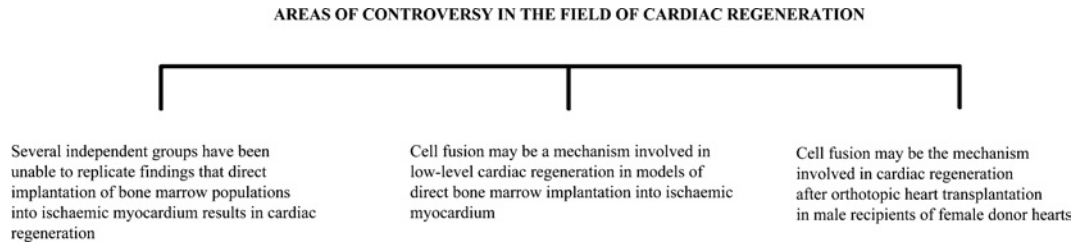
AREAS OF CONTROVERSY IN THE FIELD OF CARDIAC REGENERATION

Several independent groups have been unable to replicate findings that direct implantation of bone marrow populations into ischaemic myocardium results in cardiac regeneration

Cell fusion may be a mechanism involved in low-level cardiac regeneration in models of direct bone marrow implantation into ischaemic myocardium

Cell fusion may be the mechanism involved in cardiac regeneration after orthotopic heart transplantation in male recipients of female donor hearts

Figure 3   Schematic diagram highlighting the principal areas of controversy in the evolving field of cardiac regeneration

studied the ability of circulating bone-marrow-derived cells to regenerate myocardium after infarction using GFP$^+$ bone marrow chimaeric mice. The hearts were studied at 1 week to 2 months after LAD ligation, and they found that only rare GFP-positive cardiomyocytes (1–3 cells/100 000 cardiomycytes). The study was not designed to answer the question of whether fusion or transdifferentiation resulted in this low level of cardiomyocyte repopulation.

These three complementary studies raised many questions about the reproducibility and validity of the original study by Orlic et al. [19]. Combined, they demonstrate that local implantation of HSC-enriched bone marrow into ischaemic myocardium does not result in myocardial regeneration. Very low-level myocardial regeneration from bone marrow may occur after infarction, with or without peripheral stem cell mobilization, and this is probably a consequence of cell fusion (Figure 3). These mechanistic studies are of particular importance, given the progress of clinical trials studying the effect of bone marrow implantation after myocardial infarction in humans; they suggest that, although such treatment may be of functional benefit to patients, the mechanism for improvement is clearly not myocardial regeneration.

RESIDENT CARDIAC STEM CELLS

As investigations of bone-marrow stem cell plasticity were taking place, parallel searches for resident cardiac stem cells took place. Based on the data that low-level cardiac regeneration occurs in the heart, the hypothesis that resident stem cells exist within the myocardium was put forth. Unlike the proposal that bone marrow serves as a reservoir for cells that can differentiate into myocardium when placed in the milieu of the injured heart, this hypothesis was in keeping with the basic tenet of developmental biology that stem cells reside in the tissue that they repair.

Contributors to the original study by Orlic et al. [19] published a report in 2003 describing the isolation of adult cardiac stem cells from rat myocardium. In this study, Beltrami et al. [33] isolated a self-renewing clonogenic population of Lin$^-$c-kit$^+$ cells from normal rat myocardium. *In vitro*, these cells gave rise to cells with charac-

teristics of myocytes, smooth muscle cells and endothelial cells. *In vivo*, implantation of 1×10^5 of these cells into post-infarcted myocardium resulted in decreased infarct size, myocardial regeneration and preservation of cardiac function. The authors presented several lines of evidence to argue against cell fusion as a mechanism for myocardial regeneration. Finally, although they could not conclude whether these supposedly cardiac stem cells originally reached the myocardium through the circulation, they found no cells with similar characteristics in the bone marrow or peripheral circulation. A significant limitation of this study is that the techniques used to identify cardiac regeneration are the same as those used in the earlier study by Orlic et al. [19], and these have been called into question as multiple groups have been unable to reproduce the results [30–32].

Oh et al. [29] also report the existence of adult heart-derived cardiac progenitor cells in mice. Unlike the cells isolated by Beltrami et al. [33], these cells are Lin$^-$c-kit$^-$Sca-l$^+$; *in vitro*, 5-azacytidine can induce cardiac differentiation of these cells. Moreover, *in vivo*, these cells can differentiate into myocytes when delivered into the infarct border-zone, with approximately half of these representing fusion products between native myocytes and the donor Lin$^-$c-kit$^-$Sca-l$^+$ stem cells.

Contributors to the study by Beltrami et al. [33] also reported that, in patients undergoing aortic valve replacement for aortic stenosis, outflow tract myomectomy specimens contained clusters of stem cells making the transition to cardiomyocytes [34]. These cells were c-kit$^+$Sca-1$^+$, and some co-expressed cardiomycyte markers GATA-4 and MEF2 (myocyte-specific enhancer factor 2). They suggest that the presence of these cells strongly supports the existence of cardiac stem cells in humans. Messina et al. [35] performed studies in both mice and humans, isolating self-renewing clonogenic cells that grow as self-adherent clusters ('cardiospheres') *in vitro* and express the stem cell markers c-kit, CD-34 and Sca-1. These cells become beating cardiomyocytes when co-cultured with postnatal rat cardiomyocytes and can contribute to myocardium *in vivo* when injected into post-infarcted border-zone myocardium. More recently, Laugwitz et al. [36] described the isolation of cardiac progenitors from the heart of the postnatal rat, mouse and human which carry the genetic marker isl1 (islet-1);

Table 1   Cell types for myocardial repair

Cell type
Embryonic stem cells
Fetal cardiomyocytes
Neonatal cardiomyocytes
Adult cardiomyocytes
Bone marrow cells
Whole bone marrow
BM-MNCs
HSC-enriched bone marrow
MSCs
Endothelial progenitor cells
PB-MNCs
Endothelial cells
Smooth muscle cells
Skeletal myoblasts
Resident cardiac stem cells

these cells can adopt a fully differentiated cardiomyocyte phenotype *in vitro* in the absence of cell fusion.

In summary, these studies provide evidence that resident cardiac stem cells may exist in murine and human models. It appears that different groups of cardiac stem cells have been isolated by these researchers; notably, those of Beltrami et al. [33] are c-kit⁺, whereas those of Oh et al. are c-kit⁻. Clearly additional mechanistic studies will be needed to understand the nature and function of resident cardiac stem cells.

MYOCARDIAL CELL-BASED REPAIR

Myocardial cell-based repair has come a long way over the past decade. It is now a treatment option for end-stage heart failure patients which can be used alone or in combination with percutaneous or surgical revascularization. The discussion of the basic science behind this method of myocardial repair highlights the fact that little is known about the underlying mechanisms through which cell treatment may effect improvement in cardiac performance, and many additional mechanistic studies are needed.

Several important questions that remain unanswered regarding cell transplantation are discussed below.

What is the ideal cell type for myocardial cell-based repair?

Cell types which have been studied are listed in Table 1. Embryonic stem cells can differentiate into cardiomyocytes *in vitro* and *in vivo* [8,37], but their use is limited by ethical considerations, immunological incompatibility and the possibility of teratoma formation. Fetal and neonatal cardiomyocytes [38–40] implanted into the post-infarcted heart can form electrical connections with

native cardiomyocytes, but ethical reasons limit the use of these cell types. Adult cardiomyocytes have been used in animal models [41,42], but are not readily available for human treatment. Adult bone marrow cells, including HSC-enriched populations [19,30–32], MSCs [12,13,43–45] and endothelial progenitor cells [46,47], have been studied. The plasticity of HSC-enriched cells remains controversial, but less controversy has surrounded the ability of MSCs to differentiate into cardiomyocytes. More studies will be needed to better understand the mechanisms through which MSCs contribute to myocardium. Recently Yoon et al. [48] isolated novel adult human bone marrow stem cells that are phenotypically distinct from HSCs and MSCs, and they have shown that these clonogenic self-renewing cells are able to contribute to myocardium after infarction through both differentiation and fusion mechanisms. Several groups have reported that endothelial progenitor cells are able to repair the injured heart, and mechanisms including therapeutic angiogenesis and induction of endogenous myocardial regeneration have been proposed [46,47,49,50]. Others have reported that endothelial cells can actually transdifferentiate into cardiomyocytes both *in vitro* and *in vivo* [51]. Implantation of smooth muscle cells into the post-infarcted can result in improved cardiac performance, perhaps through changes in cardiac compliance, and has been studied in small animal models [52,53]. Skeletal myoblasts have been studied in a wide range of animal models [54–56]; myoblasts can be purified and amplified in culture from an autologous skeletal muscle biopsy. These cells strongly resist ischaemia and can then be delivered to the post-infarcted heart, where they incorporate into the myocardium and improve cardiac function. Side-by-side experiments comparing repair by MSCs and skeletal myoblasts after cryoinjury in rabbits showed similar improvement in cardiac function in both groups [57]. In fact, skeletal myoblasts were one of the first cell types to be used in human studies [58]. The MAGIC (Myoblast Autologous Graft in Ischaemic Cardiomyopathy) study is an ongoing Phase II randomized clinical trial being conducted in Europe studying the effect of skeletal myoblast transplantation into the hearts of patients with ischaemic cardiomyopathy. An earlier Phase I study demonstrated feasibility and safety, although a significant percentage of cell-treated patients experienced sustained ventricular tachycardia and required placement of internal defibrillators [59].

What is the ideal delivery method for cell-based therapy?

Several techniques for cell delivery have been proposed (Table 2), each with their advantages and disadvantages. No clear 'superior technique' has been identified. Intramyocardial injection allows for direct delivery into the damaged myocardium. This can be done through an open surgical approach (for instance, concomitantly with

Table 2 Comparison of cell-delivery methods

Method	Advantages	Disadvantages
Intramyocardial	Precise delivery to area of injury	Invasive; may increase risk of arrhythmia.
Intracoronary	Precise delivery to area of injury	Invasive; impairment of coronary flow during cell infusion.
Intravenous	Non-invasive	Requires innate homing mechanism to reach injured myocardium.

open surgical revascularization) or percutaneously (trans-endocardial delivery) using electromechanical mapping as a guide for catheter-based myocardial injections. One potential problem with direct intramyocardial injection is that it may result in islands of electrically disconjugate cells and may therefore increase the risk of arrhythmias. Intravenous injection is a second delivery method. This will only be efficacious if effective homing mechanisms exist that direct cells to the injured heart. Intracoronary injection is a third delivery method. One can deliver cells into the infarct-related artery at the time of PCI (percutaneous coronary intervention) or at a later time point, thereby optimizing the delivery of cells to the site of injury. However, impairment of coronary flow by cell delivery limits both the quantity of cells that can be delivered and the duration of the cell infusion.

What is the optimal timing for myocardial repair after infarction?

Published reports have examined the ability of cell-based therapy to effect myocardial repair both early (from time of injury to several days later) and late (several weeks later) after myocardial infarction. Because there has been little consistency in the studies, the optimal time for cell treatment remains unknown. It is likely that the optimal timing depends on the cell type used. For example, if bone marrow cells effect myocardial repair through the release of growth factors and induction of angiogenesis, their therapeutic impact may be greatest early after injury when the degree of inflammation in the infarct milieu is greatest. Other cell types may not survive in an early infarct and later delivery may be better [60].

What is the ideal number of cells to deliver during cell-based myocardial repair?

There is no consistency in published reports regarding this question, and few dose–response studies have been performed, regardless of cell type. This is clearly an area in need of much more mechanistic data.

CLINICAL STUDIES

As experimental data supporting the use of cell therapy for myocardial repair grew, early clinical studies were organized. These have included clinical studies of myoblast transplantation [59,61] and, more recently, bone marrow cell transplantation [62–76] (Table 3). We will limit the discussion to human studies of myocardial treatment with bone marrow cells.

For the most part, the studies performed have been small case series or Phase I trials examining the safety and feasibility of bone marrow cell treatment for ischaemic cardiomyopathy. Few of these studies are controlled, let alone randomized, and most are not powered to statistically assess end points such as effect on myocardial

Table 3 Clinical trials of bone marrow transplantation for IHD
*TOPCARE AMI trial; †BOOST trial; ‡MAGIC cell trial. N/A, not applicable.

Reference	Study design	Patients (n)	Treatment	Delivery method	Combined with stent or CABG?	Interval between AMI and treatment	Length of follow-up
Stamm et al. [63]	Phase I case series	12	AC133-positive cells	Intramyocardial	CABG	4–12 weeks	6–8 months
Schachinger et al. [65]	Phase I randomized controlled trial*	59	BM-MNCs vs. PB-MNCs	Intracoronary	Stent	4.9 ± 1.5 days	12 months
Tse et al. [69]	Case series	8	BM-MNCs	Transendocardial	No	N/A	3 months
Strauer et al. [70]	Phase I non-randomized controlled trial	20	BM-MNCs vs. medical therapy	Intracoronary	Stent	7–8 days	3 months
Wollert et al. [73]	Randomized controlled trial†	60	BM-MNCs vs. medical therapy	Intracoronary	Stent	4–8 days	6 months
Perin et al. [74]	Non-randomized open-label trial	20	BM-MNCs vs. medical therapy	Transendocardial	No	N/A	12 months
Kang et al. [76]	Randomized controlled trial‡	27	PCI vs. G-CSF + PCI vs. G-CSF + PB-MNCs + PCI	Intracoronary	Stent	> 2 days	6 months

function and perfusion. Many studies combine cell treatment with conventional therapy, including surgical revascularization or PCI such as angioplasty and stenting, so it is difficult to determine whether the beneficial effects seen are secondary to cell treatment or the concomitant interventions. A wide variety of cell delivery methods, cell types and cell treatment doses are used in the various studies. None of these studies determine the fate of the donor cells nor do they clearly define the mechanism whereby cell treatment may improve cardiac function or perfusion. Several of the published studies are highlighted below.

Stamm et al. [62,63] performed a safety and feasibility study of intramyocardial AC133$^+$ stem cell treatment in 12 patients with IHD undergoing CABG (coronary artery bypass grafting). They noted improved function and local perfusion, suggesting that increased angiogenesis may be the underlying mechanism. Tse et al. [69] reported a series of nine patients with IHD who were treated with intramyocardial BM-MNCs [bone-marrow-derived MNCs (mononuclear cells)] delivered with a percutaneous catheter and showed improved symptoms, myocardial perfusion and function of the ischaemic region by MRI (magnetic resonance imaging). These patients did not have concomitant CABG or PCI.

Perin et al. [74,75] performed a prospective non-randomized open-label study of BM-MNC treatment (11 patients) compared with medical therapy alone (nine patients) in patients with severe chronic IHD not amenable to PCI or surgical revascularization. Cells were delivered transendocardially into the myocardium using a percutaneous injection catheter and electromechanical mapping. At 1 year of follow-up, perfusion scanning showed greater improvement in the cell treatment group.

The MAGIC cell randomized clinical trial [76] was designed to examine the feasibility and efficacy of G-CSF therapy and subsequent intracoronary infusion of PB-MNCs (peripheral blood MNCs) in patients after AMI (acute myocardial infarction). The study was designed with three treatment arms: PCI plus G-CSF treatment alone, PCI plus G-CSF and PB-MNCs treatment, and PCI alone. Unfortunately, investigators noted a higher incidence of in-stent stenosis in patients treated with G-CSF, and therefore this study was terminated prematurely.

The BOOST (Bone Marrow Transfer to Enhance ST-elevation Infarct Regeneration) trial [73] randomized 60 patients with AMI to receive either maximal medical therapy (30 patients) or cell treatment with BM-MNCs plus maximal medical therapy (30 patients). Patients underwent PCI with stent placement for reperfusion of the infarct-related artery. Within 4–8 days, BM-MNCs were delivered through an intracoronary approach in the cell treatment group; ethical issues prevented sham interventions in the medical therapy group. At 6 months follow-up, investigators blinded to treatment status found superior global left ventricular ejection fraction in the cell-treated patients.

The TOPCARE-AMI (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction) trial [64,65] randomized 59 patients to receive either BM-MNCs (29 patients) or PB-MNCs (30 patients) in the infarct-related artery at 4.9 ± 1.5 days after AMI. Patients were compared with a matched reference group that underwent PCI alone. At 1 year of follow-up, both cell-treated groups demonstrated greater improvement in ejection fraction and viability in infarcted segments {detected by FDG-PET (2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography) scanning} when compared with the matched reference group. Two patients suffered re-infarction after cell-treatment, and the investigators could not exclude the possibility that intracoronary cell infusion contributed to these complications; however, the incidence of death and/or reinfarction in their series (3.4%) compared favourably with published data on PCI after AMI.

FUTURE DIRECTIONS

In the past decade, cell transplantation for myocardial repair has come of age; it has been translated from the bench to the bedside, and now new questions take it back to both the basic science realm and clinical setting. Although early clinical studies suggest that bone marrow transplantation into ischaemic myocardium can improve cardiac function and/or perfusion, there is an absolute need for larger randomized double-blind clinical trials to methodically assess whether cell transplantation is a useful therapy. At the same time, very little is known about the long-term fate of transplanted cells and the mechanism by which they may exert a positive effect. Early experimental studies hypothesized that myocardial regeneration by bone marrow cells occurs but, more recently, independent investigators have been unable to reproduce these data, and the concept of plasticity of the HSC has been questioned. Does bone marrow transplantation into the ischaemic heart exert its therapeutic effect through the release of soluble growth factors, induction of angiogenesis or some other mechanism? Which bone marrow cells are best-suited for transplantation? This field is rich with unanswered questions and careful science will hopefully define a therapy that improves the lives of heart failure patients.

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