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Bone Marrow Therapies for Chronic Heart Disease

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ABSTRACT

Chronic heart failure is a leading cause of death. The demand for new therapies and the potential regenerative capacity of bone marrow-derived cells has led to numerous clinical trials. We critically discuss current knowledge of the biology and clinical application of bone marrow cells. It appears unlikely that bone marrow cells can develop into functional cardiomyocyte after infusion but may have favorable paracrine effects. Most, but not all, clinical trials report a modest short- but not long-term benefit of infusing bone marrow-derived cells. Effect size appears to correlate with stringency of study-design: the most stringent trials report the smallest effect-sizes. We conclude there may be short- but not substantial long-term benefit of infusing bone marrow-derived cells into persons with chronic heart failure and any benefit observed is unlikely to result from trans-differentiation of bone marrow-derived cells into functioning cardiomyocytes. STEM CELLS *2015;33:3212–3227*

SIGNIFICANCE STATEMENT

There is considerable controversy whether hematopoietic cell transplants (often incorrectly-termed haematopoietic stem cell transplants) are effective therapy of heart disease in humans. Few publications have reviewed this topic from a stem cell biologists' point of view. In this paper we focused on biology and then critically discuss results of clinical trials.

INTRODUCTION

Chronic ischemic heart disease is a leading cause of death globally with 17 million estimated deaths each year. In the U.S. heart failure contributes to one in nine deaths [1]. The World Health Organization estimates 23 million deaths per year from cardiovascular disease by 2030 [2].

The mammalian heart has limited regenerative capacity in response to injury. Although revascularization strategies and drug therapies improved outcomes of persons with heart failure, no therapy can replace lost cardiac muscle or reverse the process. The presumed regenerative potential of stem cells (defined below) has led to clinical trials of transplants of bone marrow-derived cells to treat chronic heart disease. We review and critically evaluate these data.

STEM CELL TERMINOLOGY

The term stem cell was coined in the late 19th century to define the origin of the blood system in embryology [3]. Since then it has been used for many types of cells in developmental biology, hematology, medicine, embryology, and so forth. We previously reviewed this topic comprehensively [4]. Briefly, the term stem cell is used entirely differently in different disciplines (hematology, regenerative medicine, gerontology, gene therapy, etc.), organism being studied (*Drosophila* sp., mice, humans, etc.), context such as persistence through life, and other variables. Perhaps the most widely accepted definition of a stem cell is: "A cell which can continuously produce unaltered daughters and also has the ability to produce daughter cells that have different, more restricted properties" [5]. Recent data indicate that under special conditions it is possible to confer some or all stem cell properties on more differentiated cells which are not intrinsically stem cells. Such cells are termed induced pluripotent stem cells (iPSCs).

The term hematopoietic stem cell or mesenchymal stem cell is often used by hematologists to describe cells in the postnatal bone marrow of rodents and humans. However, mammalian bone marrow cells are heterogeneous and only a very small proportion have stem cell-like features. The estimated frequency of hematopoietic stem cells in adult human bone marrow is approximately 1 in 10^4 and of mesenchymal stem cells, 1 in 10^3.

Because of differing definitions of these cells in clinical trials we use the terms bone marrow-derived stem/progenitor hematopoietic cells (HSCs) and mesenchymal stromal/stem cells (MSCs) hereafter [4]. It should be understood most cells or combinations of cells, if any, used in these studies are not stem cells.

Cardiac Regeneration

Most data suggest muscle cells in adult mammalian heart (cardiomyocytes) lack renewal capacity. However, recent data suggest a limited but discrete capacity for myocardial regeneration after injury [6, 7]. Heart regeneration is well known in amphibians, fish, and in the mammalian developing embryo [8]. Some data suggest a very slow turnover of cardiomyocytes in humans which decreases with age [8, 9] whereas other studies report a considerable continuous cardiomyocyte renewal in humans [10, 11]. Even if these studies are correct, there is considerable debate over the origin of these newly formed cardiomyocytes [12–14].

The frequency of cardiomyocyte apoptosis in the normal human heart is estimated at approximately 1 in 10^5 cell. Assuming this process is constant and continuous and apoptosis lasts for 4 hours, 2.2% of cardiomyocytes are lost annually and approximately 95% of the cardiomyocyte mass would turn over in 30 years [12, 13]. If these assumptions and calculations are correct, a big if, there must be an efficient mechanism to generate new cardiomyocytes. Interestingly, the rate of cardiomyocyte apoptosis in persons with chronic heart failure is estimated at 80–250 times greater than in the normal heart.

Some groups attempted to answer this question by measuring the rate of DNA synthesis in human cardiomyocytes. Bergmann and coworkers measured cardiomyocyte age in humans using a 14-carbon decay approach. They estimated a cardiomyocyte renewal rate which was age-dependent, approximately 1% annually at age 20 and 0.4% at age 75. These kinetics suggest approximately 45% of cardiomyocytes are renewed over a normal human lifespan [9]. Kajstura and coworkers calculated the rate of DNA synthesis in cardiomyocytes in cadaver hearts by iododeoxyuridine incorporation. They reported high rates of cardiomyocyte labeling-indices ranging from 2.5% to 46%. Based on these data, they estimated a cardiomyocyte turn-over rate of 22% annually [14]. The difference in estimated cardiomyocytes in these studies differs approximately 50-fold and the correct rate is unknown. Some of the disparity may relate to the difficulty in distinguishing DNA repair from replication (reviewed in references [15, 16]).

Cardio-Myocyte Renewal

Several mechanisms can generate new cardiomyocytes including: (a) replication of mature cardiomyocytes [10, 17]; (b) dedifferentiation of cardiomyocytes into stem cells or progenitors [18, 19]; and (c) differentiation of resident stem/progenitor cells in cardiomyocytes [20]. Some data support each mechanism depending upon species being studies, age, experimental conditions, including mechanism of cardiac injury, measurement method, and other variables (reviewed in reference [21]). However, these data are controversial and some reports were retracted [22].

Stem/Progenitor Cells in the Heart

Several resident populations of cardiac stem/progenitor cells are reported including side-population (SP) cells, C-KIT-positive cells, SCA-1-positive, cardiospheres, Isl1-postive cells, and others (reviewed in references 21, 23). Heart or cardiac stem cells have been isolated using different approaches such as targeting surface markers (C-KIT or SCA-1) and biological features such as the ability to efflux fluorescent dye or generate multicellular spheroids (reviewed in references 24, 25). In this review, we focus on cardiac stem/progenitor cells (CSCs) with a possible bone marrow origin. Other cardiac stem cells are reviewed in reference [23].

CD117 or C-KIT is a receptor tyrosine kinase type-3 and a receptor for stem cell factor (SCF) expressed on hematopoietic stem/progenitor cells. C-KIT signaling is important in cell proliferation, differentiation, and survival [26]. Lineage (Lin)-negative C-KIT-positive stem/progenitor cells, first identified in the bone marrow, are reported to have substantial regenerative capacity and are found in human adult hearts [27, 28].

Some data suggest Lin-negative C-KIT-positive stem/progenitor cells can regenerate approximately 70% of the adult rodent heart after in vitro expansion followed by injection back into recipients with infarction [27]. However, other data suggest Lin-negative C-KIT-positive stem/progenitor cells from adult rodents do not differentiate into cardiomyocytes in vivo [29, 30]. For example, a recent study reported a minimal contribution of C-KIT-positive cells in cardiomyocyte regeneration in rodent heart. The level of C-KIT-positive-derived-cardiomyocytes was $<0.008\%$ if cell fusion is considered [31] (reviewed in reference [32]).

Stem cell antigen-1 (SCA-1)-positive cells are also present in human bone marrow [33]. Sca-1-positive cells can be isolated from human hearts and some data suggest regenerative capabilities of these cells [33]. SP cells are another cell-type initially described in human bone marrow [34]. Similar cells have been identified in human hearts. Some data suggest these cells are resident cardiac stem/progenitors cells but this is controversial.

Can Bone Marrow-Derived Stem/Progenitor Cells Become Cardiomyocytes?

A 2001 study in rodents reported LIN-negative, C-KIT-positive rodent bone marrow cells injected into the ischemic area proximal to a myocardial infarct could regenerate $>50\%$ of the infracted area in 9 days and that there was trans-differentiation of HSC into cardiomyocytes [35]. A subsequent report indicated SCF and granulocyte colony-stimulating factor mobilization of LIN-negative, C-KIT-positive in rodent HSCs significantly decreased infarct size, ventricle dilation, and diastolic stress [36]. However, the accuracy of these data was challenged by studies which reported rodent bone marrow-derived HSCs do not form cardiomyocytes when given postinfarction and that most infused HSCs differentiate into blood cells under these experimental conditions [37–39]. Other data suggest hematopoietic cells can fuse with cardiomyocytes at

low frequency [40] giving the false impression HSCs had differentiated into cardiomyocytes. In summary, there is considerable uncertainty whether bone marrow-derived HSCs can develop into functioning cardiomyocyte after infusion. Nevertheless, most studies in animal report improved cardiac function after infusing bone marrow cells. How does this happen? If improvement is not from the infused cells directly, might it be a paracrine effect (reviewed in reference [41])? We consider this possibility below.

Can Mesenchymal Stromal/Stem Cells Become Cardio-Myocytes?

Mesenchymal stem cells are a rare population of mesenchymal cells with self-renewal and differentiation properties. Mesenchymal stem cells and mesenchymal stromal cells are frequently confused with one another (they share the same abbreviation [MSC]). In most studies we discuss in this review, MSCs refer to a plastic-adherent population of cells obtained after in vitro culture. Most of these cells are mesenchymal stromal cells and not mesenchymal stem cells [4].

Studies in different species report bone marrow-derived MSCs can differentiate into cardiomyocytes in vitro after treatment with the hypomethylating drug 5-azacitidine. Under this condition, MSCs, mainly adopt myotube morphology, expressing some cardiac-specific genes (e.g., MEF-2A/MEF-2D) and peptides (e.g., myosin, desmin, actinin, and atrial natriuretic peptides) [42–45]. Coculture of human MSCs and rat neonatal cardiomyocytes results in the expression of two markers of cardiac lineage, troponin T and GATA4 but no sarcomeric organization is reported. These data suggest the cardiac microenvironment enhances the maturation of MSC into cardio-myocytes [46]. Similar data are reported after treating MSCs with cardiac tissue extracts [47], angiotensin-II [48], nitric oxide [49], salvianolic acid B [50], and micro-RNAs (miRNAs) [51–54]. However, these induced cardiomyocytes may not be functional.

Two major pathways control differentiation of MSCs into other cells, Wnt and the TGF-β superfamily [55]. Other factors including epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor may also operate (reviewed in reference 55).

miRNAs may play an important regulatory role in the heart. Some data suggest miRNAs can enhance or inhibit cardiomyocyte proliferation and survival, control postinfarct neovascularization, regulate cardiac regeneration, and inhibit cardioprotective effects of cardiac stem or progenitor cells (reviewed in references 56, 57). Combinations of miRNAs 1, 133, 208, and 499 are reported to be able to re-program cardiac fibroblasts into cardiomyocyte-like cells in vitro [58]. miRNAs 1, 16, 124, and 499 were reported to induce MSCs to form cardiomyocytes [51–54]. For example, miRNA 16 enhances G_1 phase arrest in human MSCs, contributing to their differentiation toward myogenic phenotypes in a cardiac microenvironment [51]. However, no miRNAs has been shown to operate in vivo and the regenerative capacity of these cells and clinical benefits of in vitro differentiation are unknown.

New Cardiomyocytes Versus Functional Cardiomyocytes

The target of cell therapy is generating mature functioning cardiomyocytes which replace the damaged myocardium and improve cardiac function. This goal is challenging and many obstacles must be overcome. Most replacement strategies are based on providing immature cardiomyocytes or committed progenitors. Maturation and specification toward subtypes of cardiomyocytes such as ventricular or atrial cardiomyocytes require an understanding of the molecular pathways of cardiac subtype specification and maturation.

Even if cardiomyocytes could be generated, the electrophysiological behavior of these cells needs to be controlled. Distribution of gap junctions plays a role in cardiomyocyte coupling and electrical conduction in the heart. In humans, neonatal ventricular cardiomyocytes are confined primarily to intercalated discs for longitudinal conduction. As the heart matures these neonatal cardiomyocytes must acquire the adult pattern of gap junction distribution for the heart to function normally. Most data suggest new cardiomyocytes derived from adult bone marrow cells may not acquire this adult distribution and resemble neonatal cardiomyocytes [59, 60]. Furthermore, mismatches in cell size and shape, macroscopic myocardial fiber alignment, persistent automaticity of generated cardiomyocytes, and immaturity in intracellular calcium handling capacity are necessary for new cardiomyocytes to function properly. Whether this can be achieved is unknown even if new cardiomyocytes can be generated (reviewed in reference [60]).

Possible Mechanisms by Which Bone Marrow Cells Might Improve Cardiac Function

As we discussed, initial reports suggested bone marrow-derived cells trans-differentiated into cardiomyocytes. More recent data suggest trans-differentiation to cardiomyocytes is rare and that other mechanisms may operate such as cell-fusion. However, this also is a rare event [37, 38, 61, 62]. Amplification of vascular endothelial cells and neovascularization is another possible mechanism of improving cardiac function by bone marrow-derived cells. For example, some in vitro studies report the potential of MSCs cell to differentiate into endothelial cells [63]. Some studies in rats and dogs report increased expression of CD31, von Willebrand factor, and smooth muscle (SM)-actin and increased capillary density in MSCs after transplant, resulting in improved cardiac performance [64, 65]. In the rat, transplanted allogeneic MSCs are detectable in infarcted myocardium for up to 6 months and express markers suggesting muscle and endothelium phenotypes [66]. MSCs may be attracted to the infarct zone, possibly mediated by pleiotropic factors such as stromal cell-derived factor-1α through activation of the PI3K/AKT pathway [67]. The hypoxic environment of the infarct zone increases vascular endothelial growth factor, hepatocyte growth factor, and insulin-like growth factor 1 expression by transplanted MSCs which may contribute to angiogenesis by inducing proliferation of endothelial cells and α-SM actin-positive cells [68]. In a pig model of chronic ischemic cardiomyopathy, allogeneic MSCs were reported to differentiate into cardiomyocytes, SM cells, and endothelial cells [69]. However, these results could not be replicated in a dog model [64].

Numerous paracrine effects of bone marrow cells, alone or combined, are alleged to improve cardiac function including: (a) immune-modulation; (b) altered CSCs metabolism; (c)

stimulation of resident CSCs; (d) recruitment of endothelial progenitor cells; (e) increased contractile capabilities of residual cardiomyocytes; (f) antiapoptosis; (g) altered cell-cell interactions; (h) cardiac niche reconstitution; (i) inhibition of cardiac fibroblasts; (j) altered extracellular matrix; (k) decreased arrhythmias; and (l) increased nerve regeneration. The sheer number of suggested mechanisms suggests none is convincingly proven (reviewed in references [41, 68]; Figure 1).

Clinical Trials

Chronic heart disease results from loss of contractile cardiomyocytes. Different types of cells have been evaluated for their ability to replace this loss, including skeletal myoblasts, bone marrow-derived cells, cardiac stem cells, and mesenchymal stem cells [70]. Studies in animals, reviewed above, suggested that infusing (often referred to as transplanting) autologous or genetically identical bone marrow-derived cells could improve chronic heart failure. Mechanisms by which this improvement might occur are reviewed above. These observations led to many clinical trials of this approach in persons with acute and chronic heart disease from diverse causes. Here, we critically review trials done to improve chronic heart failure (Tables 1–3). Trials of this approach in acute heart failure are reviewed elsewhere [71–73].

Methods

Evaluating results of clinical trials of cell therapy of chronic heart disease requires analyzing the source, composition, and route of delivery of the cell product. In most studies, 80–250 ml of bone marrow is aspirated from the subject's iliac crest and the mononuclear cell fraction isolated [72]. Sometimes CD133-postive or CD34-postive cells are selected by immunological methods such as monoclonal antibodies attached to magnetic beads.

The method and route of cell delivery is a crucial variable in analyzing results of clinical trials (reviewed in reference [72]). Current techniques are depicted in Figure 2 [72]. Intravenous injection is a relatively inefficient route because few infused cells reach the infarct site [72, 121]. Normal coronary blood flow is 80 ml/minute which is about to approximately 3% of cardiac output. Consequently, there would need to be many circulation passages for most or all the injected cells to reach an infract-related artery (assuming there was perfusion, direct or collateral, to the infarct zone). An alternative, presumably more efficient route is intracoronary injection. PET scanning after this approach shows a cardiac retention rate of $<$3% [122]. During intracoronary artery injection cells are typically injected into the perinecrotic area of the infarct-related artery. During this time of vessel occlusion, cells are infused via the intracoronary route through the balloon. Percutaneous transluminal coronary angioplasty prevents backflow of cells and produces a stop flow beyond the site of balloon inflation to facilitate migration of cells into the infarct zone [72]. In some studies adhesion molecules are introduced to facilitate homing of infused cells to the site of ischemia-reperfusion injury [123].

Another approach involves trans-endocardial intramyocardial injection into the ischemic area. This approach appears safe in chronic ischemic heart disease and intractable angina (see Tables 1–3). However, orientation by electro-mechanical mapping requires technical expertise and cell loss into the ventricle, incorrect injections sites, ventricular arrhythmias, and cardiac tamponade may occur [72].

Epicardial intramyocardial injection is sometimes done into exposed ischemic areas, allowing for multiple injections within and around the infarct area. This approach is usually done concurrently with coronary artery bypass graft (CABG) (see tables). Injections are performed after the graft coronary artery anastomosis is complete, the ischemic area visualized, and cells injected into the border zone of the infarct [72].

Trials Using Bone Marrow-Derived Mononuclear Cells

Bone marrow mononuclear cells are heterogeneous populations of lymphocytes, monocytes, endothelial progenitor cells, mesenchymal stromal/progenitor cells, and hematopoietic stem/progenitor cells. Most clinical trials of bone marrow mononuclear cells were done in subjects with acute, not chronic, heart disease. The few with chronic heart disease report short-term safety and improvement in cardiac function and quality of life (Table 1).

Recently, van Ramshorst et al. reported a randomized double-blinde clinical trial of intramyocardial injection of bone marrow mononuclear cells in subjects with chronic myocardial ischemia refractory to medical treatment. There was approximately a 3% absolute increase in left ventricular ejection fraction (LVEF) after 3 months, improved MRI-derived and tissue Doppler imaging-derived parameters of diastolic function, and a substantial increase in quality-of-life score [86, 93]. In another study with chronic heart failure subjects received intracoronary bone marrow mononuclear cells after treatment of the heart with low-energy ultrasound shock waves. The authors reported a modest, but statistically significant increase in LVEF and a decrease in the number of major adverse cardiac events [95].

Perin et al. reported using trans-endocardial injection of autologous bone marrow mononuclear cells into the peri-infarct viable myocardium using a specialized (NOGA Myostar) catheter in subjects with chronic heart failure (CHF) [75, 124]. They reported improved LVEF, reduced end-systolic volume, improved cardiac performance assessed by SPECT, increased myocardial perfusion, and exercise capacity compared with controls [75, 94, 125]. In another study where the injection was into the scar tissue and in which the authors reported improved early outcomes, no benefit in late outcomes was detected including percent myocardial defect, total defect size, fixed defect size, regional wall motion, or clinical improvement in the cell therapy arm [73, 94].

Trials of Bone Marrow-Derived CD133$^+$ Cells

CD133 antigen (prominin-1) is a trans-membrane glycoprotein expressed on immature hematopoietic stem/progenitor cells. Some data suggest CD133 may be an alternative marker to CD34 which is commonly used to identify hematopoietic stem/progenitor cells [125]. Results of studies using CD133-positive cells to treat chronic heart failure are similar to those

Table 1. Clinical trials using bone marrow mononuclear cells

	Number of patients	Disease	Study	Randomized	Route	Results	Cells dose	Follow-up
Silva et al. [74] U.S.-Brazil	5	Severe ischemic heart failure	Prospective, open-label	No	Trans-endocardial with electromechanical mapping	Safely, improved exercise capacity	N/A	6 months
Perin et al. [75] U.S.-Brazil	20 Subjects ($n = 11$) Controls ($n = 9$)	Ischemic cardiomyopathy	Controlled prospective, open-label	No	Trans-endocardial with electromechanical mapping	Improve myocardial perfusion and exercise capacity	Each specific cell phenotype calculated 10(3) cells/mm^2	6–12 months
Beeres et al. [76] (J Nucl Med.) The Netherlands	25	Refractory angina	Open-label Uncontrolled	No	Intramyocardial	Increased LV function, enhanced myocardial stress perfusion in injected segments, no change in size of myocardial scar tissue	10E+8	6 months
Beeres et al. [77] (Am J Cardiol) The Netherlands	20	Severe angina and stress-induced myocardial ischemia	Open-label uncontrolled	No	Intramyocardial	Less angina, improved exercise capacity, no ventricular arrhythmias, improved myocardial perfusion, increased LVEF	N/A	6 months
Beeres et al.[78] (Am Heart J.) The Netherlands	25	Refractory angina with chronic myocardial ischemia	Open-label uncontrolled	No	Intramyocardial	Safe, decreased angina, improved myocardial perfusion, LVEF and QoL	10E+8	12 months
Beeres et al. [79] (Heart Rhythm J) The Netherlands	20	Refractory angina with myocardial ischemia	Open-label uncontrolled	No	Intramyocardial	No increase in ventricular arrhythmias, no change in electrophysiological properties, improved LVEF	N/A	6 months
Beeres et al. [80] (Heart J) The Netherlands	15	Ischemia on SPECT and CCS class III or IV angina	Uncontrolled Open-label	No	Trans-endocardial	Feasible, safe, clinical improvement, decreased angina, improved LVEF and myocardial perfusion	10E+8	6 months
de la Fuente et al. [81] Argentina	10	Chronic post-MI heart failure patients who are transplant candidates-	Uncontrolled Open-label	No	Trans-endocardial	Safe, improved LVEF	86 × 10E+6	12 months
Nasseri et al. [82] Germany	10	end-stage heart failure with mechanical VAD	Uncontrolled Open-label	No	Intramyocardial	no increase the likelihood of successful weaning from VAD support	52–164 × 10E+7	range 24–498 days
Yelda et al. [83] Turkey	10	End-stage ischemic cardiomyopathy	Prospective, open-label	No	Intracoronary	Improved symptoms, LVEF and perfusion	36 × 10E+6	6 months

Table 1. *Continued*

	Number of patients	Disease	Study	Randomized	Route	Results	Cells dose	Follow-up
Beeres et al. [84] The Netherlands	24	Chronic refractory angina and chronic myocardial ischemia	Uncontrolled Open-label	No	Intramyocardial	Improved MRI and TDI-derived parameters of diastolic function	10E+8	3 months
van Ramshorst et al. [85] Heart J The Netherlands	24 Experimental ($n = 14$) Control ($n = 10$)	Severe post-MI heart failure	Controlled	No	Intramyocardial	Improved LVEF, less LV dys-synchrony, increased global strain	10E+8	3 months
van Ramshorst J et al. [86] JAMA The Netherlands	50 Experimental ($n = 25$) Control ($n = 25$)	Chronic myocardial ischemia refractory to medical treatment	Randomized, double-blind, placebo-controlled	Yes	Intramyocardial	Modest improvement in myocardial perfusion	10E+8	3 months
Gao et al. [87] China	60 Experimental ($n = 20$) Control ($n = 20$) Normals ($n = 20$)	MI-induced heart failure	Controlled	–	Intracoronary	Increased apelin	24–48 × 10E+6	21 days
Pokushalov et al. [88] Russia	109 Experimental ($n = 55$) Control ($n = 54$)	Chronic ischemic heart disease	Randomized	Yes	Intramyocardial	Safe, improved survival, clinical symptoms and LV function	N/A	12 months
Willerson et al. [89] FOCUS trial U.S.	87 Experimental ($n = 58$) Controls ($n = 29$)	CAD and LV dysfunction with angina or symptomatic heart failure	Randomized, phase-2	Yes	Trans-endocardial	See Perin et al. [94]	–	5 years
Pokushalov et al. [90] Russia	CRT + Cell therapy: 26	Ischemic heart failure LVEF <35%, LBBB and mechanical dys-synchrony	Randomized, single-blind, crossover	Yes	Intramyocardial BMMC and CRTD system implantation	Improved LV performance in combination of cell therapy and CRT	N/A	1 year
Hu et al. [91] China	60 Experimental ($n = 31$) Control ($n = 29$)	CABG candidates+ chronic heart failure	Randomized, double-blind, placebo-controlled	Yes	CABG ± BMMC	Improved myocardial function	10E+7	6 months
de la Fuente et al. [81] TABMMI trial Argentina	20	Post-MIheart failure with EF ≤ 40%	Open-label phase-1	No	Trans-endocardial	Safe, increased EF and ETT	10E+8	2 years
Rodrigo et al. [92] The Netherlands	16	Chronic myocardial ischemia prior intramyocardial	Randomized, crossover	Yes	Intramyocardial	Improved myocardial perfusion, QoL	10E+8	6 months
van Ramshorst [93] Circ Cardiovasc Imaging The Netherlands	50 Experimental ($n = 25$) Control ($n = 25$)	Chronic myocardial ischemia	Randomized, double-blinded, placebo-controlled	Yes	Intramyocardial	Improved MRI-derived and TDI-derived parameters of diastolic function	10E+8	3 months

Table 1. *Continued*

	Number of patients	Disease	Study	Randomized	Route	Results	Cells dose	Follow-up
Perin et al. [94] JAMA FOCUS-CCTRN trial U.S.	153	Ischemic cardiomyopathy	Phase-2 randomized double-blind, placebo controlled trial	Yes	Trans-endocardial	No improvement in LVESV, maximal oxygen consumption or reversibility on SPECT	10E+8	6 months
Assmus et al. [95] CELLWAVE trial Germany	103 Experimental ($n = 82$) Control ($n = 21$)	CHF post-MI	Double-blind, randomized, placebo-controlled trial	Yes	Intracoronary	Modest improvement in LVEF	N/A	4 months
Honold et al. [96] (Clin Cardiol) China	157	CHF	Uncontrolled	No	Intracoronary	24 patients died (15.2%),superior prognostic power of the SHFM score compared to CPET	N/A	4 years
Honold et al. [97] (Clin Res Cardiol) China	124/154	CHF	Uncontrolled	No	Intracoronary	Improved exercise capacity	N/A	3 months

Abbreviations: CABG, coronary artery bypass graft; CAD, Coronary artery disease; CCS, Canadian Cardiovascular Society; CHF, Chronic heart failure; CPET, Cardiopulmonary exercise testing; CRT, cardiac resynchronization therapy; EF, ejection fraction; LBBB, Left Bundle Branch Block; LV, left ventricle; LVEF, left ventricular ejection fraction; MI, myocardial infarction; SHFM, Seattle heart failure model; SPECT, single-photon emission computed tomography; TDI, tissue Doppler imaging; VAD, ventricular assist devices.

Table 2. Clinical trials using bone marrow CD 133+ cells

	Number of patients	Etiology of heart failure	Type of study	Route of injection	Results	Cell dose	Follow-up duration
Pompilio et al. Ann Thorac Surg [98] Italy	4 patients	-A large ischemic area in the left ventricle -Refractory angina	Pilot, open-label uncontrolled	Intramyocardial	Safe, feasible, and reproducible procedure	13.7 × 10E+6 −200 × 10E+6	4 months
Hendrikx et al. [99] Belgium	20 CABG only: n =10 CABG + cell injection: n =10	Post-MI heart failure candidates for CABAG	Randomized controlled clinical trial	Intramyocardial	No difference in LVEF but a recovery of regional contractile function in previously nonviable scar	60 × 10E+6	4 months
Goussetis et al. [100] Greece	8	Chronic ischemic cardiomyopathy	Pilot, open-able, uncontrolled	Intracoronary	Significant number of cells attracted to spleen, liver and retained myocardium	1.5 × 10E+6 CD133+ and CD133-CD34+ selected	–
Klein et al. [101] U.S.-Germany	10	End-stage chronic ischemic cardiomyopathy (ejection fraction <22%)	Pilot, open-label, uncontrolled	Intramyocardial	Feasible and safe Improved LVEF	1.5–9.7 × 10E+6 cells	9 months
Manginas et al. [102] Greece	24 Cell therapy: n = 12 Control group: n = 12	Patients with old, nonviable anterior myocardial infarction	Pilot, single center, controlled	Intracoronary	Sustained improvement in segmental myocardial perfusion and in favorable left ventricular remodeling	64%, $CD133^+$ + 76% $CD133^-CD34^+$	28.0 ± 8.7 months
Ahmadi et al. [103] Iran	27 CABG only: n = 9 CABG + cell injection: n = 18	CABG candidates	Prospective, nonrandomized, open-label study	Intramyocardial	Some clinical improvement	1.89 × 10E+6	6 months
Yerebakan et al. [104] Rostock trial Germany	32 patients	Chronic myocardial ischemia	Pilot	Intramyocardial	Safety after long-term follow-up Rostock trial	–	5 years
Stamm et al. [105] Germany	43 CABG only: n = 21 CABG + cell injection: n = 22	CABG candidates	Randomized controlled clinical trial	Intramyocardial	Safety, improved LVEF	5.8 × 10E+6	6 months

Table 2. *Continued*

	Number of patients	Etiology of heart failure	Type of study	Route of injection	Results	Cell dose	Follow-up duration
Pompilio et al. [106] Italy Thorac Cardiovasc Surg	5	Severe chronic angina	Pilot, open-label, uncontrolled	Intramyocardial	– Improvements of rest and stress perfusion in the injected territories – Increase in the collateral score in angiography	– Mobilized BM-Cells: $n = 3$ – Iliac crest BM-Aspirate: $n = 2$ 4–12 × 10E+6	1 year
Yerebakan et al. [107] J Thorac Cardiovasc Surg Germany	35 Cell therapy: $n = 26$ Control group: $n = 14$	MI-induced heart failure undergoing CABG	Controlled trial	Intramyocardial injection during CABG	Preoperative LVEF and time since myocardial infarction may be critical for patients selection and benefit from cell therapy	6 × 10E+6	36 months
Donndorf et al. [108] Trial J PERFECT Trial Germany	142	Chronic ischemic heart disease patient undergoing CABG	Randomized, double-blind, placebo controlled, multicenter trial	Intramyocardial injection during CABG	Ongoing	0.5–5 × 10E+6	6 months
Forcillo et al. [109] IMPACT-CABG Canada	5	Chronic ischemic cardiomyopathyin patients undergoing CABG (IMPACT-CABG)	Open-label pilot study	Intramyocardial	– Initial safety and feasibility – Improved quality of life – No improvement in LVEF	8 × 10E+6	18 months

Abbreviations: CABG, coronary artery bypass graft; MI, myocardial infarction.

Table 3. Clinical trials using bone marrow-derived MSCs

	Number of patients	Etiology of heart failure	Type of study	Route of injection	Type of cells	Results	Number of cells	Follow-up duration
Mohyeddin-Bonab et al. [110] Iran	8	Old myocardial infarction versus control group	Open label, controlled	-Intramyocardial during CABG: $n = 5$ -Intracoronary duringPCI: 3	Autologous ex vivo expanded MCSs	Safe and feasible procedure, improved cardiac function	5.55 × 10E+6	6 months
Trachtenberg et al. [111] TAC-HFT trial U.S.	60 patients – Group A: 30 receive MSCs injection or placebo (2:1 ratio) – Group B: 30 BMCs or placebo (2:1 ratio)	Post-MI CHF LVEF:15%–50%	Randomized, double-blind, placebo-controlled study	Trans-endocardial	MSCs [CD105,CD45 CFU] versus whole bone marrow mononuclear cell	See Heldman et al. [118]	200 × 10E+6	12 months
Zeinaloo et al. [112] Iran	1	11-Year-old children with dilated cardiomyopathy	Case report	Intracoronary	Autologous ex vivo expanded mesenchymal stromal/progenitor cells	Myocardial biopsy 8 months after the procedure showed widening of the interstitium with increased number of interstitial cells, clinical improvement	4.8 × 10 E+6/ml × 6	12 months
Hare et al. [113] POSEIDON trial U.S.	30 patients (5 patients in each cell type per dose level)	30 patients with LV dysfunction due to ICM	Randomized trial	Trans-endocardial	Ex vivo expanded BM-derived mesenchymal stromal/progenitor cells Allogeneic versus autologous	– Allogeneic and autologous MSCs reduced mean EED by sphericity index but did not increase EF – MSC injection improved patient functional capacity, quality of life, and ventricular remodeling	20 × 10E+6, 100 × 10E+6, 200 × 10E+6	1 year
Mathiasen et al. [114] MSC-HF Trial Denmark	60 (2:1 pattern of MSCs or placebo)	Chronic ischemic heart failure	Randomized, double-blind, placebo-controlled trial	Intramyocardial	autologous Ex vivo expanded mesenchymal stromal/progenitor cells	See Mathiasen et al. [120]	12–15 × 10 E+6	12 months
Mathiasen et al. [115] MSC-HF Trial Int J Cardiol Denmark	31	Severe stable coronary artery disease	Open-label uncontrolled	Intramyocardial	Autologous ex vivo expanded mesenchymal stromal/progenitor cells (cell culture expanded and stimulated with VEGF to facilitate endothelial differentiation)	– Improved exercise time and angina class – quality of life – Highly reduced admission rates for stable angina, revascularization and overall cardiovascular disease – Improved symptoms and slowing down disease progression	21.5 × 10 E+6	3 years

Table 3. *Continued*

	Number of patients	Etiology of heart failure	Type of study	Route of injection	Type of cells	Results	Number of cells	Follow-up duration
Bartunek et al. [116] C-CURE trial Belgium	Control: $n = 15$ Experimental: $n = 32$	Prospective, multicenter, randomized trial	Randomized, prospective, open-label	Endo-myocardial	Isolated cardiopoietic mesenchymal stem cells	Cardiopoietic stem cell therapy was found feasible and safe with signs of benefit in chronic heart failure, meriting definitive clinical evaluation	733 × 10E+6	24 months
Karantalis et al. [117] PROMETHEUS trial U.S.	6	Chronic ischemic cardiomyopathy not receiving bypass graft for clinical	Pilot	Intramyocardial injection into akinetic/ hypokinetic myocardial territory	Mesenchymal stem cells	Comprehensive regional functional restitution, which drives improvement in global LV function	–	18 months
Heldman et al. [118] TAC-HFT trial U.S.	65 patients – MSC arm ($n = 19$) with placebo ($n = 11$) – BMCs arm ($n = 19$) with placebo ($n = 10$)	Ischemic cardiomyopathy and LV ejection fraction less than 50%	A phase 1 and 2 randomized, blinded, placebo-controlled study	Transendocardial	Autologous MSCs [CD 105, CD45 CFU] versus whole bone marrow mononuclear cell	– 6-Minute walk distance increased with MSCs arm only – Quality of life improvement MSCs and with BMC but not with placebo – Infarct size reduced in MSC arm but not in BMC or Placebo – Regional myocardial improved with MSCs group but not BMCs or placebo – Left ventricular chamber volume and ejection fraction did not change	200 × 10E+6	12 months
Brahmanandam et al. [119] U.S.	1	Light-chain amyloidosis heart failure	Pilot	N/A	Autologous cell transplantation	Improved clinical status	N/A	2.5 years
Mathiasen et al. [120] MSC-HF Trial Denmark	55 patients	Severe ischemic heart failure	Randomized, double-blind, placebo-controlled	Intramyocardial	MSC or placebo	Significant improvements in LVEF of 6.2% and stroke volume	53.8 × 10E+6	6 months follow-up

Abbreviations: BMC, bone marrow cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; LV, left ventricle; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MSC, mesenchymal stromal/stem cell; VEGF, vascular endothelial growth factor.

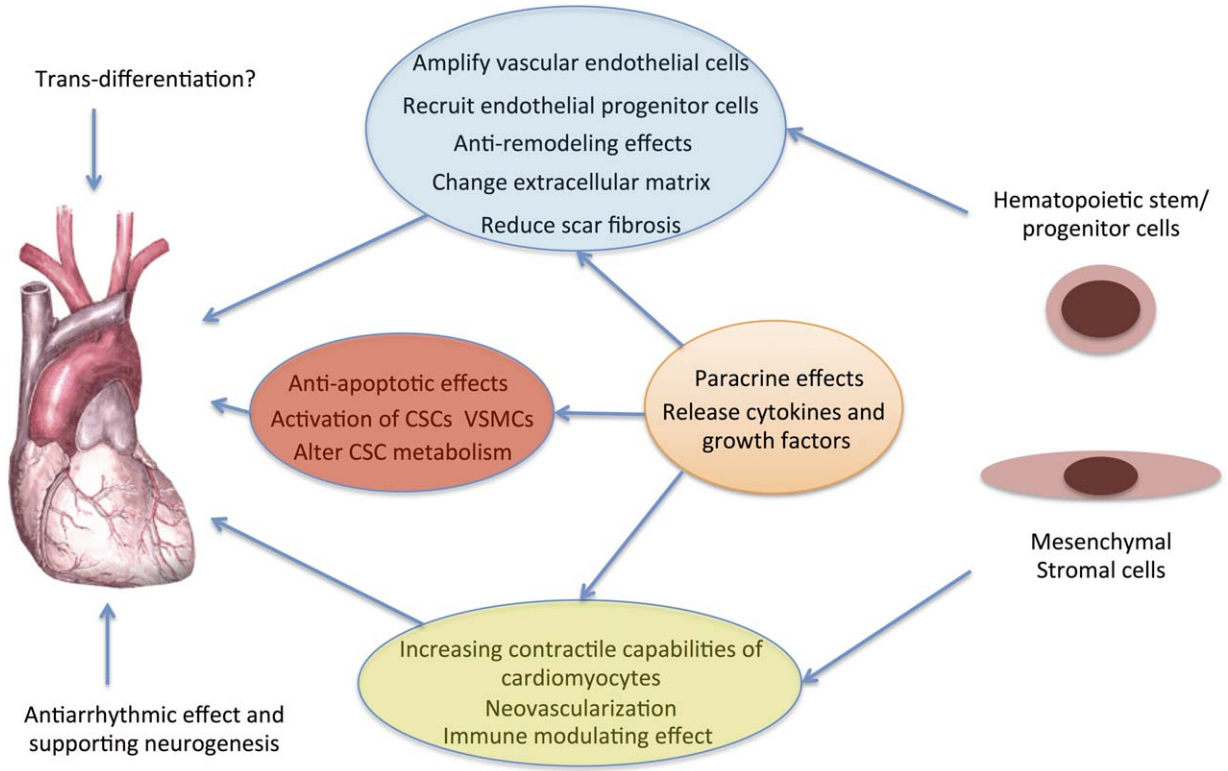

Figure 1. Possible mechanisms by which bone marrow-derived cells may contribute in cardiac regeneration. Abbreviations: CSCs, cardiac stem/progenitor cells; VSMCs, vascular smooth muscle cell.

reported with bone marrow mononuclear cells. Initial studies reported safety and feasibility of intramyocardial injection of these cells (Table 2). In one randomized trial, CD133-positive cells were injected into the infarct border zone during CABG [105]. LVEF was moderately but significantly increased at 6 months in the cell therapy cohort. However, the small size of

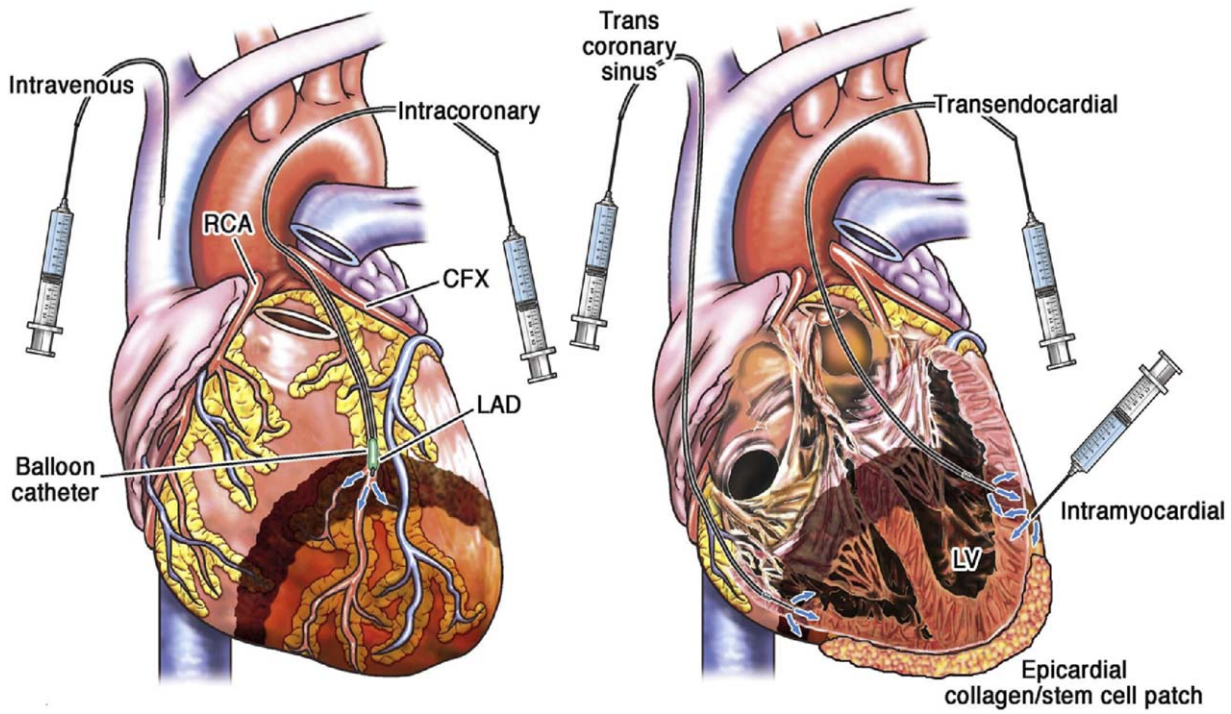

Figure 2. Routes of injections for cardiac cell therapy. Abbreviations: CFX, circumflex artery; LAD, a left anterior descending artery; LV, left ventricle; RCA, right coronary artery (adopted from reference [72] with permission).

this study limits the conclusion [105]. Another randomized, double-blind, placebo controlled multicenter clinical trial of 142 subjects is likely to be published soon [108].

Trials of Bone Marrow-Derived MSCs

Results of studies of MSCs are summarized in Table 3. In one study, 60 subjects were randomized to receive a trans-endocardial injection of MSCs, bone marrow mononuclear cells, or a placebo. Serious adverse events were similar in the cohorts. Better quality of life was reported in both cell therapy cohorts compared with placebo. Improvement in 6-minute walk distance, reduction in infarct size, and improved regional myocardial function were detected only in subjects receiving MSCs. However, there was no change in ventricular chamber volume or ejection fraction [118]. In another randomized and blinded study, MSCs were expanded in vitro and partially differentiated toward cardiomyocytes before injection. Most injected cells were MEF-2C-(myocyte-specific enhancer factor 2C)-positive, a marker of heart cells. These investigators reported improved LVEF (from 27.5% to 34.5%; $p < .0001$) and an increase in the 6-minute walk at 6 months [116]. However, there are discrepancies in various reports of this study [126]. Although the authors corrected some errors, these data have not been replicated independently [127].

In another study, five subjects received trans-endocardial injections of MSCs. Thirty additional subjects were then randomized to receive trans-endocardial injections of autologous versus allogeneic MSCs. Both cohorts showed reduced infarct size and improved ventricular remodeling compared to baseline. A comparison of function of injected versus noninjected area of the myocardium showed small scar size in all segments, especially near injected sites. The greatest improvement was seen in severe segmental left ventricular dysfunction [128]. Only the allogeneic MSC cohort had reduced left ventricle end-diastolic volumes whereas paradoxically, improved 6-minute walk distance and quality of life score were mainly observed in the autologous MSC cohort [113]. However, there is not placebo control in this study.

Conclusions

There is progress, albeit small, after more than a decade of preclinical and clinical studies of using bone marrow-derived cells to treat chronic heart failure. It is now reasonably certain bone marrow-derived cells do not differentiate to cardiomyocytes and that the benefit of this intervention, if any, is on cardiac function from paracrine effects of the infused or injected cells. Most studies, but not all, report a small improvement in heart function but appropriate controls are often lacking. There are some biases, inconsistencies, and discrepancies in reporting (reviewed in reference [71]) and a trend of decreasing effect-size with increasingly stringent study-designs. For example, we found the magnitude of benefit measured by an increase in LVEF is inversely related to study-design stringency with the more carefully designed studies showing the smallest effect-size (Fig. 3). There are also many unknowns such as whether any cell type or delivery route is better, or whether live cells are needed. If benefit is predominately from a paracrine effect perhaps injecting intact living cells versus cell extracts or dead cells versus conditioned medium or other bioactive agents would work as well. These are important questions for the future.

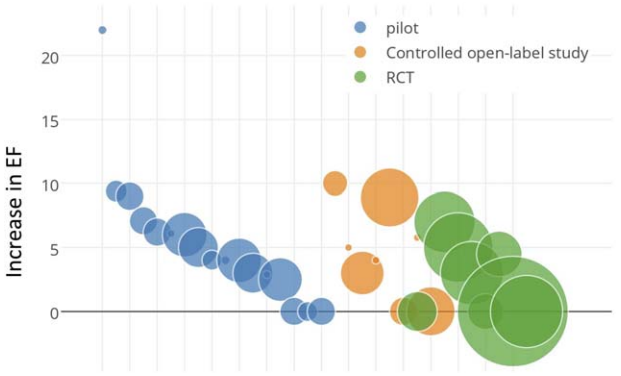

Figure 3. Increase in left ventricular ejection fraction after cell therapy in chronic heart failure studies according to the type of study (the size of circle represents the number of patients in each study). Abbreviation: EF, ejection fraction.

Perhaps more promising areas for near future studies to treat chronic heart failure involve development of mechanical replacement hearts, cardiac scaffolds (such as with three-dimensional-printing and the biomembranes of cardiomyocytes) and use of iPSCs-derived cardiomyocyte. More research is needed.

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Author Contributions

I.S.B., A.K., and R.P.G.: conception, design, research, writing, approval of final manuscript, and financial support.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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