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Review

Potential benefits of cell therapy in coronary heart disease

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ABSTRACT

Cardiovascular disease is the leading cause of morbidity and mortality in the world. In recent years, there has been an increasing interest both in basic and clinical research regarding the field of cell therapy for coronary heart disease (CHD). Several preclinical models of CHD have suggested that regenerative properties of stem and progenitor cells might help restoring myocardial functions in the event of cardiac diseases. Here, we summarize different types of stem/progenitor cells that have been tested in experimental and clinical settings of cardiac regeneration, from embryonic stem cells to induced pluripotent stem cells. Then, we provide a comprehensive description of the most common cell delivery strategies with their major *pros* and *cons* and underline the potential of tissue engineering and injectable matrices to address the crucial issue of restoring the three-dimensional structure of the injured myocardial region. Due to the encouraging results from preclinical models, the number of clinical trials with cell therapy is continuously increasing and includes patients with CHD and congestive heart failure. Most of the already published trials have demonstrated safety and feasibility of cell therapies in these clinical conditions. Several studies have also suggested that cell therapy results in improved clinical outcomes. Numerous ongoing clinical trials utilizing this therapy for CHD will address fundamental issues concerning cell source and population utilized, as well as the use of imaging techniques to assess cell homing and survival, all factors that affect the efficacy of different cell therapy strategies.

Contents

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Introduction

Despite the fact that the incidence of cardiovascular disease (CVD) has dramatically declined over the past four decades, due to the remarkable advances in the understanding of CVD pathophysiology and treatment, coronary heart disease (CHD), stroke, and peripheral arterial disease (PAD) remain the leading causes of death and disability in Western countries [1]. In particular, myocardial infarction (MI) is associated with elevated mortality and morbidity because it causes heart failure (HF) by inducing cardiomyocyte (CM) death and apoptosis. Until now, reducing the established risk factors for CVD has worked successfully for both secondary and primary prevention. However, a novel and complementary approach, which could represent a major breakthrough in the field, would be the possibility to repair the damaged myocardium and/or blood vessels. This opportunity originates from the possibility of applying cell therapy to CVD and even more from the revolutionary concept that the human heart is not a post-mitotic organ, as traditionally believed, but an organ capable of regenerating its damaged and/or aged structures thanks to the activity of recently discovered endogenous or exogenous adult stem cells capable of improving tissue repair through regeneration of vessel and cardiac muscle cells [2]. Indeed, a burst of regenerative activity was observed in the heart of one-day-old mice after resection of the left ventricular apex [3].

Recent attempts based on transplantation of adult stem and progenitor cells to damaged areas of the cardiovascular system have produced interesting and promising results. However, to date there is still the need to address some fundamental unresolved issues, such as identifying the optimal cell type, delivery strategy, therapeutic dose and timing, as well as determining the extent of cell survival and retention in the different settings. In this context, current preclinical studies are aimed at ameliorating homing, cell survival, and retention and they are essentially based on genetic modifications and the use of biomaterials for cell delivering. It is crucial that ongoing and future clinical studies address these essential issues.

Here, we review the clinical aspects of different cell therapy strategies in patients with CHD; we will also focus on the current, both completed and ongoing, clinical trials utilizing bone marrow cells (BMCs) as source for cell therapy.

Stem cell sources for cardiovascular regenerative therapy

Cell therapy for CHD can be different according to the disease progression, but it is invariably expected to provide a renewable source of proliferating, functional CMs. However, stem cells are rare in humans: approximately, only 1 out of 10,000–100,000 BMCs has been recognized as a hematopoietic stem cell, and only 1 out of 30,000 cells in the heart has been identified as a c-kit-positive cardiac stem cell (CSC) [4]. Cardiac niches have been identified that provide a harboring microenvironment to support and protect CSCs, as well as control their turnover and migration toward sites of myocardial injury [4]. Interestingly, canine pulmonary veins have been shown to host cardiac stem cell niches [5].

In both preclinical and clinical studies, different types of cells have been employed for cell therapy, mainly varying in regard to their origin, expression of surface markers, function, and ability to derive different cell types (Fig. 1a–d).

Embryonic stem cells

Embryonic stem cells (ESCs), deriving from the embryo inner mass at the blastocyst stage, would be the ideal stem cells. These totipotent cells display the maximum potential for organ regeneration and can differentiate into a variety of cell types and tissues, including CMs and blood vessels, but they also increase the risk of teratoma formation [6,7]. In several animal models ESC transplants improved cardiac function [8] or blood perfusion [6,9]. Furthermore, genetically engineered human ESCs were able to electrically pace quiescent, recipient ventricular CMs *in vitro* and ventricular myocardium *in vivo* [10]. The advantage of using ESCs derives from their unlimited proliferative capacity and multilineage differentiation plasticity, whereas the main disadvantages are the social and ethical concerns due to the source and isolation methods. This ethical matter, together with their potential genetic instability and consequent risk of cancer development, renders these cells not suitable for clinical application.

Fetal stem cells

As regard to cell source, fetal and human umbilical cord blood cells should have more plasticity than adult stem cells even though their pluripotency degree after *in vitro* expansion is still unclear. These cells, comprising hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), and somatic stem cells with proliferative capacity, have showed promising results in animal models, but no clinical studies were available until recently [11].

Adult stem cells

Bone marrow cells

Although the ideal cell type for CHD cell therapy remains to be determined, most clinical trials refer to the use of adult stem cells from autologous bone marrow (BM) as the main source of adult stem cells for cardiac regeneration. Despite the more limited differentiation capacity, this choice can be explained by several reasons, which include their easy availability and safety, the possibility to expand and/or select them *in vitro*, the nonnecessity of an immunosuppressive treatment, and the lack of ethical controversies associated with the use of embryonic stem cells. Thus, most of the published studies have utilized these cells (see Tables 1 and 2).

BM contains different cell subpopulations that have the potential to migrate to distant sites and differentiate into cells with diverse phenotypes [12]. BMCs may be isolated by direct aspiration or by mobilization into the peripheral blood through the use of cytokines such as granulocyte-colony stimulating factor (G-CSF) [13,14]. The two main groups of BM-derived stem cells are the HSCs and the mesenchymal stem cells or MSCs, which can be further

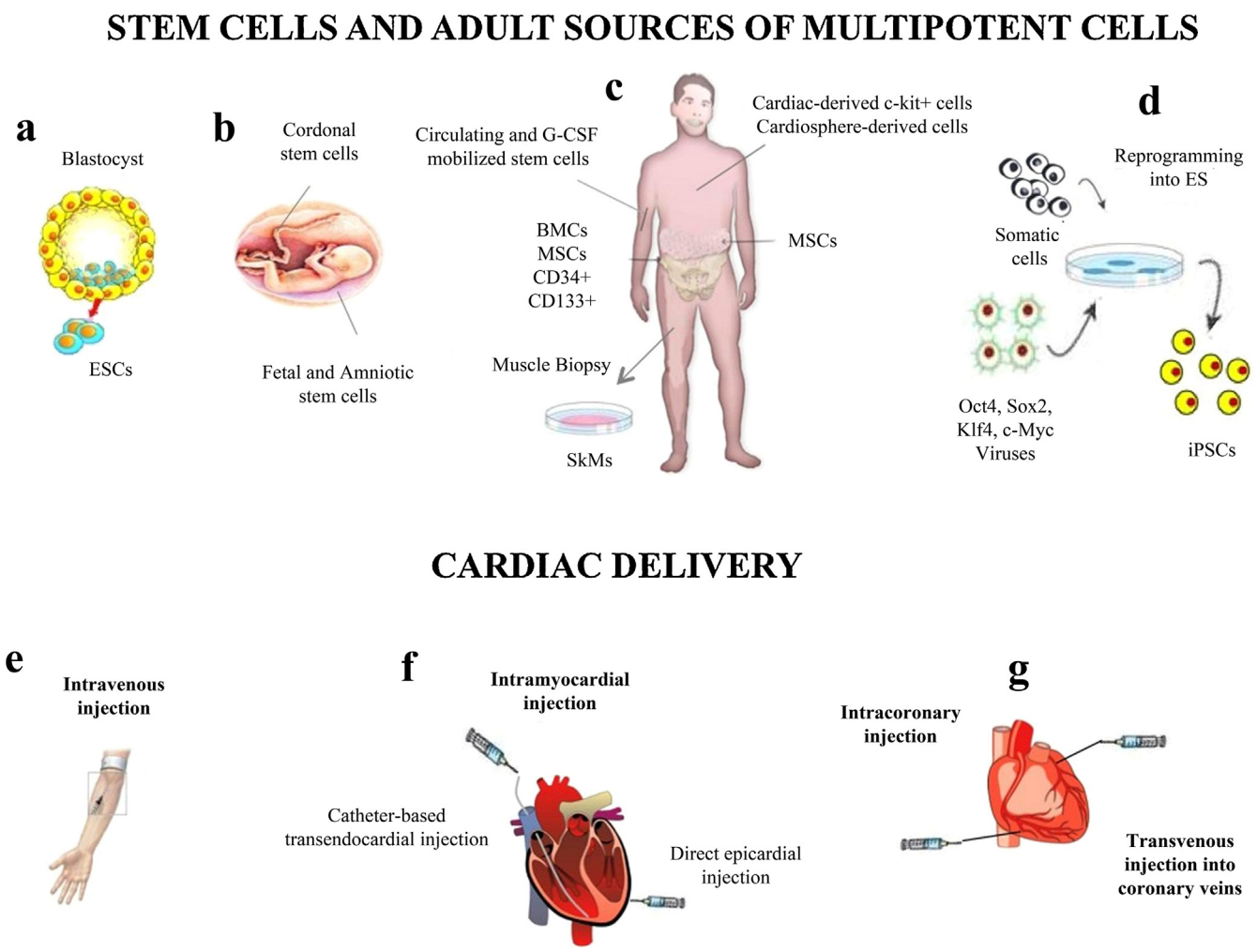

Fig. 1. (a–d) Stem cells and adult sources of multipotent cells for therapeutic intervention in coronary heart disease; embryonic stem cells (a), fetal and amniotic stem cells (b), adult multipotent stem cells (c), and induced pluripotent stem cells (d). (e–g) Cell delivery strategies used in cell therapy of heart diseases; intravenous injection (e), intramyocardial injection (f), intracoronary injection (g). BMCs, bone marrow cells; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells; SkMCs, skeletal muscle cells.

subdivided into different subpopulations according to the expression of specific cell surface receptors.

The HSCs are usually identified by the expression of CD34, CD45, and/or CD133 antigens and have been widely studied and clinically used for a variety of disorders. A heterogeneous cell subpopulation includes endothelial progenitor cells (EPCs), which are found also in peripheral blood and fetal liver or umbilical cord blood. These cells are defined by the co-expression of the HSC markers CD34, CD45, or CD133 and the vascular endothelial growth factor receptor-2 (VEGFR2/KDR), although further cell populations expressing monocyte markers have been also identified [15,16]. Importantly, both EPC populations are known to directly or indirectly contribute to angiogenesis and promote neovascularization [15,16]. The use of isolated circulating EPCs, including G-CSF-mobilized BM progenitors, has been extensively treated in a recent review [15] and it will not be the object of this paper.

The multipotent, non-hematopoietic MSCs originate from the mesoderm and the neuroectoderm and reside mainly in BM, besides liver, white adipose tissue, intestine, lung, periodontal ligament, and dental pulp [17]. The isolation of highly purified MSCs is based on their surface antigen profile; indeed, these cells are positive for CD73, CD90, and CD105, and negative for CD34, CD45, CD14/CD11b, CD79α/CD19, and HLA-DR antigens [17]. The potential regenerative role is mainly based on experimental animal studies since to date there are few published clinical studies with isolated MSCs [17,18]. Indeed, in phase I clinical trials of acute MI, MSC therapy has been found to improve left ventricular function, induce reverse remodeling, and decrease scar size, thus suggesting their utilization in future clinical practice [17,18]. These cells are present also in other adult tissues, such as adipose tissues, and they can trans-differentiate into functional CMs and a variety of other cells [19–21]. Clinical trials are now ongoing to investigate the safety, feasibility, and efficacy of adult stem cells isolated from adipose tissue in patients with both acute and chronic myocardial ischemia.

In the end, a great deal of evidence documents that transplanted BMCs reduce infarct size and improve left ventricular function and perfusion in experimental animal models of acute MI and ischemic cardiomyopathy [22]. However, available human studies demonstrate only a modest improvement in functional and structural parameters in patients with both acute MI and chronic ischemic heart disease, compared to conventional state-of-the-art therapy [23].

Skeletal myoblasts

Further adult stem cells sources have been tested for their therapeutic potential. For example, skeletal myoblasts have been injected into the ischemic myocardium; however, neither

Table 1
Published heart disease studies.

NIH registration number	Study type	No. of treated patients	Cell type	Condition	Delivery route	Year [Ref]
NCT00114452	Randomized; controlled; blinded	39	MSCs	MI	IV	2009 [18]
NCT00199823	Randomized; controlled	50	BMCs	AMI	IC	2006 [44]; 2008 [45]; 2009 [46]
NCT00264316	Randomized; controlled; blinded	33	BMCs	AMI	IC	2006 [47]
NCT00289822	Randomized; controlled	28	BMCs	MI	IC	2006 [48]
Unregistered	Randomized; controlled	29	BMCs	AMI	IC	2002 [49]; 2004 [50]; 2011 [51]
Unregistered	Randomized; controlled	30	BMCs	MI	IC	2004 [52]; 2006 [53]; 2009 [54]
NCT00279175	Randomized; controlled; multicenter	101	BMCs	AMI	IC	2006 [55]; 2006 [56]; 2010 [57]
Unregistered	Randomized; controlled	24	BMCs	NICM	IC	2006 [58]
Unregistered	Randomized; controlled	17	BMCs	AMI	IC	2007 [59]
Unregistered	Randomized; controlled; blinded	33	BMCs	AMI	IC	2009 [60]
Unregistered	Randomized; controlled	40	BMCs	AMI	IC	2009 [61]
NCT00316381	Randomized; controlled; multicenter	80	CD34+ from BMCs	AMI	IC	2009 [62]
NCT00284713	Randomized	33	BMCs	NICM	IC	2009 [63]
Unregistered	Randomized; controlled	191	BMCs	CIHD, HF	IC	2010 [64]
NCT00235417	Non-randomized	32	BMCs	CIHD, HF	IC	2010 [65]
NCT00268307	Randomized; controlled; blinded	30	BMCs	MI	IC	2010 [66]
NCT00313339	Randomized; controlled	16	BMCs	STEMI	IC	2011 [67]
NCT00669227	Randomized; controlled; blinded	29	BMCs	AMI	IC	2010 [68]
NCT00400959	Randomized; controlled	15	CD133+ from BMCs	STEMI	IC	2011 [69]
NCT00395811	Randomized; controlled; blinded	31	BMCs	MI	IC	2011 [70]
NCT00200707	Randomized; controlled; multicenter	52	BMCs	AMI	IC	2011 [71]
NCT00684060	Randomized; controlled; blinded; multicenter	58	BMCs	MI	IC	2011 [72]
Unregistered	Randomized; controlled	10	BMCs	ICM, CHF	IM	2005 [73]
Unregistered	Randomized	10	SkMs	MI	IM	2005 [26]
Unregistered	Randomized; controlled	10	BMCs	MI	IM	2006 [74]
Unregistered	Randomized; controlled	12	SkMs	MI	IM	2006 [28]
Unregistered	Randomized; controlled	18	BMCs	MI	IM	2006 [75]
Unregistered	Randomized; controlled	20	CD133+ from BMCs	CIHD	IM	2007 [76]
Unregistered	Randomized; controlled	18	BMCs	IHF	IM	2008 [77]
Unregistered	Randomized; controlled	34	SkMs	LVD, MI	IM	2008 [25]
Unregistered	Randomized; controlled	25	BMCs	ICM	IM	2009 [78]
Unregistered	Randomized; controlled	12	SkMs	ICM	IM	2009 [27]
Unregistered	Randomized	12	MBCs	CAD	IM	2010 [79]
Unregistered	Randomized	27	BMCs	CAD	TEC	2006 [80]
Unregistered	Randomized	10	BMCs	AMI, RA	TEC	2006 [81]
Unregistered	Non-randomized	10	BMCs	MI	TEC	2007 [82]
Unregistered	Randomized; controlled; blinded	19	BMCs	CAD	TEC	2007 [83]
Unregistered	Randomized; controlled	50	BMCs	MI	TEC	2009 [84]
NCT00203203	Randomized; controlled	20	BMCs	HF	TEC	2011 [85]
NCT00824005	Randomized; controlled; blinded	92	BMCs	ICM, CIHD	TEC	2012 [86]

AMI, acute myocardial infarction; BMCs, bone marrow cells; CAD, coronary artery disease; CHF, congestive heart failure; CIHD, chronic ischemic heart disease; HF, heart failure; IC, intracoronary injection; ICM, ischemic cardiomyopathy; IHF, ischemic heart failure; IM, intramyocardial injection; IV, intravenous injection; LVD, left ventricular dysfunction; MI, myocardial infarction; MSCs, mesenchymal stem cells; NICM, nonischemic cardiomyopathy; NIH, National Institutes of Health; RA, refractory angina; SkMs, skeletal myoblasts; STEMI, ST elevation myocardial infarction; TEC, transendocardial injection.
Detailed information about the NIH registered clinical trials are reachable visiting the web site http://clinicaltrials.gov/.

clear evidence of transdifferentiation into CMs, nor improvement in the patient outcome have been observed (see Table 1) [24–28]; recently, the use of "second generation" skeletal myoblasts through cell enrichment methods has been hypothesized [29]. On the other side of the coin, epicardial injection of skeletal myoblasts was associated with malignant arrhythmias [30].

Resident cardiac stem cells

Resident CSCs have been identified in the hearts of adult humans and other mammalian species, thus revolutionizing the long-believed concept of the heart as a post-mitotic organ [4,31]. These cells represent an autologous source of CSCs with the additional advantage of being tissue-specific and pre-committed to the heart lineages. However, the limited number of resident CSCs imposes their expansion before they can be used to achieve therapeutic goals. This has been accomplished by inducing *in situ* proliferation of resident CSCs with appropriate stimuli. Despite their high proliferative potential, their capacity to repair extensive injury, as in the case of acute MI, is still uncertain [4,31]. Animal studies using CSCs have demonstrated inconsistent beneficial effects [4,32]. However, initial results from a randomized phase I trial suggest that intracoronary infusion of autologous CSCs is effective in improving left ventricular (LV) systolic function and reducing infarct size in patients with heart failure after MI, and warrant further, larger, phase II studies [33]. Yet, recently, the safety and efficacy of intracoronary cardiosphere-derived cells in regenerating infarcted myocardium has also been shown by the CADACEUS trial, a randomized controlled prospective two-center trial in subjects with LV dysfunction 2–3 months post-MI [34]. As in most of these studies, also this trial did not include a placebo control group because of the treatment invasiveness. Thus, further studies with a larger number of treated subjects are required to demonstrate the safety and efficacy of these therapeutic strategies.

Finally, it is important to recall that intramyocardial injection of c-kit$^+$ BMCs can induce endogenous progenitor-derived CMs resulting in improved ventricular function in mice after MI [35].

Table 2
NIH registered ongoing clinical studies with a number of heart disease patients > 50.

NIH registration number	Status	No. of treated patients	Cell type	Condition	Delivery route	Phase
NCT00877903	Active, not recruiting	220	MSCs	AMI	IV	II
NCT00326989	Unknown	100	BMCs	CHF	IC	I and II
NCT00437710	Unknown	50	BMCs	AMI	IC	I and II
NCT01350310	Recruiting	60	BMCs	HF	IM	II
NCT01267331	Recruiting	60	BMCs	CMI, LVD	IM	I and II
NCT00711542	Recruiting	100	BMCs	MI	IC	I and II
NCT00790764	Active, not recruiting	60	BMCs	HD, CAD	IC *vs* TEC	II
NCT00418418	Unknown	60	MSCs	HF, MI, CAD	IM	II
NCT00644410	Not yet recruiting	60	MSCs	CHF	IM	I and II
NCT00936819	Not yet recruiting	100	EPCs from BMCs	MI	IC	II
NCT00462774	Completed	60	CD133+ from BMCs	CAD, CHF, MI	IM	II and III
NCT00950274	Recruiting	142	CD133+ from BMCs	MI, CAD	IM	III
NCT00810238	Active, not recruiting	240	BMCs	HF	TEC	II and III
NCT00984178	Unknown	120	BMCs	AMI	IC	II
NCT00384982	Completed	116	BMCs	MI	Percutaneous, IM and IC	II
NCT01392105	Completed	80	MSCs	AMI	IC	II and III
NCT00691834	Not yet recruiting	50	BMCs	AMI, HF	IC	II
NCT00908622	Recruiting	50	SkMs	MI	Percutaneous implantation	II
NCT00725738	Unknown	80	BMCs	AMI	IC	II and III

AMI, acute myocardial infarction; BMCs, bone marrow cells; CAD, coronary artery disease; CHF, congestive heart failure; CIHD, chronic ischemic heart disease; EPCs, endothelial progenitor cells; HF, heart failure; IC, intracoronary injection; ICM, ischemic cardiomyopathy; IHF, ischemic heart failure; IM, intramyocardial injection; IV, intravenous injection; LVD, left ventricular dysfunction; MI, myocardial infarction; MSCs, mesenchymal stem cells; NICM, nonischemic cardiomyopathy; NIH, National Institutes of Health; RA, refractory angina; SkMs, skeletal myoblasts; STEMI, ST elevation myocardial infarction; TEC, transendocardial injection.
Detailed information about the NIH registered clinical trials are reachable visiting the web site http://clinicaltrials.gov/.

Induced pluripotent stem cells

To overcome the ethical issue of ESCs, interest has focused on induced pluripotent stem cells (iPSCs), which display ESC-like properties, but originate from adult somatic cells through nuclear reprogramming by ectopic expression of stemness factors [36–38]. Novel and improved approaches of generating iPSCs by virus-free gene delivery methods have been recently developed in order to achieve patient-specific stem cells [39]. Another issue to be considered is that iPSCs maintain the epigenetic memory of the cell type from which they derive. The clinical potential of these cells remains to be determined, even though recent studies have demonstrated that human iPSCs can differentiate into functional myocytes [40]. Thus, iPSCs may represent a good promise in the field of regenerative medicine.

Future directions

Epigenetic control of CM differentiation

Increasing evidence supports the role of epigenetic mechanisms in regulating the switch between maintenance of stemness and lineage commitment, including the transition from cardiac progenitor cells toward CMs [41]. The recent discovery that a long noncoding RNA is crucial in the cardiovascular lineage commitment during mammalian development highlights new avenues for the differentiation of novel CMs [42]. It is predictable that in the near future it will be possible to drive cardiac cell differentiation back and forth by manipulating the epigenetic background of CMs and their precursors for regenerative purposes.

Fibroblasts transdifferentiation into CMs

Both mouse embryonic fibroblasts and postnatal cardiac or dermal fibroblasts have been successfully transdifferentiated into functional CMs, following forced expression of specific transcription factors [43]. The possibility of reprogramming fibroblasts into cardiomyocytes directly within the infarct scar is on the horizon.

Cell delivery strategies

A safe, effective and feasible delivery system is essential for the efficacy of cardiovascular regenerative therapy. The objective of cell therapy, regardless of the cell source, is to repair the damaged tissue by delivering the adequate cell number to the interested area. The specific delivery technique should also elicit little or no risk of hematogenous dissemination, since progenitor cells are expected to cause metastatic tumor formation in the long-term. For this reason, local administration methods should be favored instead of systemic delivery methods. Moreover, optimal results are achieved when cells are retained at the delivery site. Factors influencing delivery include the microenvironment at the delivery site, which is crucial for cell survival, maintenance, and/or homing.

Several cell delivery strategies are currently available for different cell types and target anatomical sites, ranging from direct intramyocardial injection to intravascular, catheter-based, methods (Fig. 1e–g). However, as discussed below, none of them can now be considered the preferred method.

The four major techniques used to deliver stem and progenitor cells directly into the myocardium include: systemic intravenous injection, intracoronary injection, and intramyocardial injection (both direct epicardial injection and catheter-based transendocardial injection) (Fig. 1e–g) (see Table 1) [18,25–28,44–85]. Variations within each approach have been developed to solve issues of safety, feasibility, cell viability, and retention. Moreover, further strategies, such as intrapericardial delivery, are also under study [86]. The schedule of cell therapy related to the onset of disease is also a crucial issue, especially for ischemic disease. The advantages and disadvantages of the different strategies are briefly discussed below.

Intravenous delivery

The intravenous delivery through a central venous catheter would be the easiest and least invasive method, but currently, due to many disadvantages, it is not the method of choice in clinical studies for heart diseases. Indeed, the efficacy of intravenous delivery of stem cells is mostly limited because of the entrapment of the injected cells in the lungs and other organs [87]. A clinical study of intravenous infusion of BM-derived MSCs after MI has shown the safety of intravenous infusion of MSCs after 12-month follow-up

and preliminary data about the efficacy of this therapy are available [18]. Presently, a phase II multicenter study is ongoing to evaluate the safety and efficacy of intravenous infusion following acute MI (NCT00877903).

Intracoronary delivery

Nowadays, most studies are performed through percutaneous cell delivering, either by intracoronary or transendocardial methods, the safety of which has been clinically demonstrated [44–46,52–57,83–85]. In the intracoronary delivery approach, cells are injected under pressure into a coronary artery through a balloon catheter placed in the coronary artery, with the advantage that the cells can be directly applied at the occlusion site, for example after an acute MI. The pioneer clinical trial of intracoronary infusion of mononuclear BMCs was performed on 20 patients and established that such practice was safe and effective [49]. Thereafter, TOPCARE studies have demonstrated long-term safety after 5-year follow-up and suggested favorable effects on LV function [50,51]. Moreover, additional recently published clinical trials (see Table 1) have confirmed that intracoronary infusion is a safe strategy with a modest cardiac function improvement [65–72]. Conversely, intracoronary transfer of autologous BMCs did not augment recovery of global LV function in patients with timely reperfused MI, but it was found to favorably affect infarct remodeling in a randomized, double-blind, controlled study [47]. In the group of negative studies, the results of the ASTAMI trial indicate that intracoronary BMC treatment in acute MI is safe in the long term, although only a small improvement in exercise time was found in the treated group [46]. Moreover, other studies did not show positive effects of intracoronary cell delivery in patients with MI [68] or they found several complications [59], thereby suggesting that, despite the easy applicability, this approach presents also some disadvantages. However, the study of Penicka had several limitations [59], such as the small number of enrolled patients that also had poor LV function and late revascularization, thus suggesting that this subset of patients could not benefit from the autologous bone marrow cell transplantation. Differently, in the BOOST trial, although a substantial improvement in LV ejection fraction was observed at 6 months [52], a subsequent analysis at 18 months [53], and a further evaluation at 5 years [55], have shown that a single dose of BMCs was not able to promote a sustained improvement in LV systolic function. Moreover, no significant differences in mortality and other clinical endpoints between groups have been observed at the longest follow-up [54]. However, this trial was not powered to assess clinical outcome; so these data can only support the safety of the procedure, but not the possible effects on subsequent clinical events. More recently, the results of the rigorously designed LateTIME study have been published [72]. The novelty of this trial was to investigate the use and therapeutic efficacy of intracoronary autologous BMC delivery 2–3 weeks following MI whereas the majority of published trials have administered BMCs within the first week following primary percutaneous coronary intervention [72]. Finally, intracoronary administration of BMCs improved global LV-function and ameliorated adverse LV remodeling also during long-term follow up after MI in the REPAIR-AMI study [55–57,88].

Although there are few studies investigating non-ischemic cardiomyopathies, pilot studies indicate that intracoronary BMC implantation can have potential clinical benefits also in these patients [58,63].

Controlled Phase III clinical trials (NCT01392105, NCT00725738) are currently undergoing to evaluate the clinical effects of intracoronary delivery of BMCs on cardiac function (see Table 2).

Transendocardial delivery

The transendocardial method allows direct cell delivery into the target regions, also in patients with occluded arteries, and usually involves the use of a customized injection catheter. Delivery can also be facilitated by the guidance of electromechanical mapping to identify with more precision suitable myocardial regions within the ischemic area [89]. Alternatively, fluoroscopic-based guidance for cell delivery can be used to this aim. This approach can be a good choice for patients with chronic heart disease, whereas it may be not appropriate for patients with acute MI because of the potential risk of endocardial damage or ventricular perforation [89]. These observations have also been demonstrated in preclinical studies [89]. Several clinical trials of transendocardial injection have been published [79–85]. More recently, a randomized clinical study of transendocardially-injected BMCs in severe coronary artery diseases demonstrated that this strategy could improve cardiac function [79].

Intramyocardial injection

Autologous BMCs have been delivered intramyocardially during surgical interventions, such as coronary artery bypass grafting surgery in patients with chronic ischemic heart disease [76]. Several clinical studies have suggested that direct myocardial cell implantation can be an effective treatment for patients with chronic ischemic HF [73,76,77,85]. Indeed, this approach provides a direct route of cells delivery with good chance of cell engraftment in the damaged area. A potential problem can be the increased number of early postoperative arrhythmic events, as shown in a study combining myoblast injections with coronary surgery; however, these events may be also related to the cell type and/or disease context [25]. Although cell therapy combined with surgical intervention may provide additional benefits in the future, it is difficult to evaluate the overall effects of this clinical strategy. Another catheter-based direct method using the coronary system, during coronary artery bypass grafting, was shown to be safe and feasible in most patients; moreover, it was possible to reach otherwise inaccessible areas of the cardiac apex [26]. This approach has displayed great potential in patients with ischemic heart disease even though there are not additional studies supporting these promising results.

Clinical studies comparing the different cell delivery techniques are lacking. A small study compared intracoronary artery delivery with retrograde coronary venous approach in patients with ischemic heart disease. This study demonstrated higher cell retention in ischemic myocardium when the intracoronary approach was used, although the small sample size and the characteristics of study design did not permit the authors to reach conclusions on this important issue [90]. However, preclinical studies have shown that intracoronary and transendocardial injection of BMCs resulted in increased engraftment when compared with intravenous infusion. Moreover, intracoronary delivery was more efficient than transendocardial delivery, but it was also associated with decreased coronary blood flow, thereby suggesting that local transendocardial delivery may be a preferable method for cell delivery [91].

Independently of the cell-delivering strategy utilized, the different outcomes observed in all the clinical studies with bone marrow-derived progenitor cells may be related to differences in cell preparation protocols [92] as well as to imaging techniques for ejection fraction evaluation by ventriculography, magnetic resonance imaging, and echocardiography [23,92].

The efficiency of delivery and retention is generally lower than expected, and retention and survival of cells at target sites are still inadequate. Indeed, a low rate of progenitor cell survival has been found after injection of BMCs into the infarcted heart with

different delivery strategies, either by intracoronary delivery of autologous BMC transplantation [93] or direct myocardial injection in a preclinical rat model [94]. Moreover, the efficiency seems to be independent of the cell type; for example, similar discouraging results were also found for skeletal myoblasts implanted into mouse hearts [95]. The cause of transplanted cell death is probably related to the nature of ischemic tissue and the presence of endogenous factors, such as inflammatory molecules. Thus, approaches to ameliorate survival of delivered cells are still under investigation.

Paracrine hypothesis

Human studies of cell therapy in CHD have shown an overall improvement in cardiac and vascular function other than tissue regeneration; however, in many cases the observed effects cannot be explained by the efficiency of stem and progenitor cell delivery and engraftment. Thus, the alternative paracrine hypothesis has been recently advanced and should be considered in the evaluation of the therapeutic efficacy of all these studies. This hypothesis establishes that the injected cells act in a paracrine manner by releasing soluble factors into the surrounding tissue, thus contributing to cardiac repair and regeneration. These paracrine factors, such as cytokines, chemokines, and growth factors, are able to induce myocyte protection, CM cell cycle re-entry, improved cardiac metabolism, upregulation of angiogenesis and neovascularization, and they may mediate endogenous regeneration through recruitment and activation of resident stem cells [96]. The demonstration and the whole understanding of these paracrine mechanisms can have many future clinical implications in the field of cell therapy for CHD. Indeed, the use of paracrine factors improving cardiovascular regeneration could be a safer treatment, due to a lower risk of tissue disruption compared to direct cell injection. Finally, it has also been hypothesized that an ideal regenerative therapy would employ a combination of both cells and paracrine factors.

Conclusions and future perspectives

Cell therapy for CHD has a great therapeutic potential. Several clinical trials, mainly phase I/II studies, have demonstrated the safety and efficacy of adult stem and progenitor cells in regenerative therapy of the cardiovascular system. However, some issues, such as cell source, dose, timing and delivery method, remain to be optimized, and various phase III clinical trials are now ongoing also to address these points (see Table 2). As previously discussed, several types of cell sources are now under investigation and include cells derived from embryos (ESCs), fetal and umbilical cord blood cells, as well as adult stem and progenitor cells isolated from bone marrow, and also from other tissues such as adipose tissue, and, finally, reprogrammed cells (iPSCs).

Despite major progress in cell delivery to the damaged heart, better cell engraftment and higher cell survival remain the major problems of current procedures especially for treatment of large damaged areas and congenital heart defects. Another crucial point for the efficacy of cell therapy in restoring cardiovascular function is the three-dimensional reorganization of cells in the injured region and the correct orientation of contracting elements.

Studies investigating the extent of cell homing and the long-term engraftment, using *in vivo* imaging techniques, revealed that only a low percentage of cells could be detected both in animal models and in clinical trials; these findings suggested that a better understanding of homing mechanisms might be crucial for enhancing cell engraftment especially when cells are infused *via* the vascular route [97]. In turn, the poor engraftment causes either limited regeneration of cardiac tissue and/or blood vessels and also reduces the paracrine activity, which is known to significantly contribute to repair processes. Homing to damaged sites is a complex process involving the interaction of several chemokines, chemokine receptors, intracellular signaling, adhesion molecules (selectins and integrins), and proteases [98]. Basically, two strategies might be used to enhance cell homing: cell pre-treatment to activate incorporation or target tissue pre-treatment to provide cytokines and chemo-attractant factors capable to stimulate cell engraftment [97].

Novel approaches aimed at improving cell survival are under study and they include cell preconditioning [99], microencapsulation [100], and genetic modification [101]. Also, alternative strategies involving tissue engineering are now under investigation to ameliorate the efficacy of cell therapy [89,102]. The leading approaches consist of the use of injectable matrices as vehicles for cell delivery; examples are given by collagen, engineered myocardial patch made of viable and autologous tissue, matrigel with ESC-derived CMs alone and cultured with endothelial cells with or without mouse embryonic fibroblasts [89,102]. A different method involves the utilization of the so-called “cell sheet engineering” for myocardial regenerative therapy using temperature-responsive culture dishes, which can be set with a single type of cells, such as monolayered MSCs, myoblast or ESC-derived cardiac progenitors, or as a composite cell culture, with CMs, endothelial cells, and fibroblasts [89,102].

Tissue engineering can provide natural and/or synthetic matrices favoring the survival, proliferation, and differentiation of the implanted cells. Moreover, these scaffolds can drive the appropriate cell alignment, supporting the restoration of the structural and mechanical features of the native cardiac tissue.

Despite promising results in animal models, standard cell therapies still have limited clinical applications. Probably, a great chance for successful cell-based therapies in CVD could arise from the joint effort of cell biology and tissue engineering.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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