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REVIEW ARTICLE

The role of stem cells in treating coronary artery disease in 2018

Robert E. Michler[1,2]

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Abstract

The last decade has witnessed the publication of a number of stem cell clinical trials, primarily using bone marrow-derived cells as the injected cell. Much has been learned through these "first-generation" clinical trials. The advances in our understanding include the following: (1) cell therapy is safe; (2) cell therapy has been mildly effective; and (3) human bone marrow-derived stem cells do not transdifferentiate into cardiomyocytes or new blood vessels. The primary mechanism of action for cell therapy is now believed to be through paracrine effects that include the release of cytokines, chemokines, and growth factors that inhibit apoptosis and fibrosis, enhance contractility, and activate endogenous regenerative mechanisms through endogenous circulating or site-specific stem cells. The current direction for clinical trials includes the use of stem cells capable of cardiac lineage.

Keywords Heart failure · Cardiac stem cells · Bone marrow mononuclear cells

Introduction

Over one million lives are lost each year to myocardial infarction (MI) in the USA and countless more from its consequence, heart failure [1]. Indeed, it is estimated that 1% of the Western world carries the diagnosis of heart failure, and in the USA alone, approximately five million Americans currently live with the disease, and an additional 400,000 patients are newly diagnosed each year [2].

Many heart failure patients are ineligible for heart transplantation or mechanical circulatory assistance and remain without a viable medical, interventional, or surgical treatment option. It has been estimated that over 100,000 patients annually in the USA may be in this "no-option" group [3]. It is precisely this population of patients for whom stem cell regenerative therapy may be applicable.

✉ Robert E. Michler
rmichler@montefiore.org

[1] Department of Surgery, Montefiore Medical Center, Albert Einstein College of Medicine, Greene Medical Arts Pavilion 5th Floor, 3400 Bainbridge Avenue, New York City, NY 10467, USA

[2] Department of Cardiothoracic & Vascular Surgery, Montefiore Medical Center, Albert Einstein College of Medicine, Greene Medical Arts Pavilion 5th Floor, 3400 Bainbridge Avenue, New York City, NY 10467, USA

The aspirational goal of stem cell therapy is the treatment of myocardial infarction by regenerating cardiomyocytes and blood vessels, and as a result, improving cardiac function. Regenerative surgery is the transplantation of immature progenitor cells into a region of infarction with the expectation that these cells will produce new blood vessels and cardiac muscle cells. Preclinical studies in animal models have demonstrated profound regeneration of new blood vessels and cardiomyocytes and increased left ventricular (LV) function [4]. In 2018, this goal in human clinical trials is far from an established reality.

More than 3000 patients worldwide have been enrolled in clinical trials involving just bone marrow cells (BMCs). The majority of these "first-generation" clinical trials have involved some form of bone marrow-derived cells, despite the unambiguous knowledge that BMCs do not normally form cardiomyocytes. It has been hypothesized that BMCs might be coaxed in humans to form cardiomyocytes and new blood vessels, as they have been in rodents. Clinical studies have not yet yielded the expected results, and in fact, clinical trials have been mostly disappointing with only modest, transient, and inconsistent improvement in symptoms, LV function, and LV geometry.

Nevertheless, these first-generation clinical trials have been very informative and cell therapy has been shown to be feasible and safe. There is no evidence in humans for true cellular regeneration, that is, the development of new cardiomyocytes or

new blood vessels that unequivocally have arisen from transplanted stem cells. Whatever clinical benefit that has been seen is most likely the consequence of paracrine effects resulting from the release of cytokines, chemokines, and growth factors, which activate endogenous reparative mechanisms, inhibit apoptosis and fibrosis, and enhance contractility.

Types of stem cells

Stem cells are defined by having specific characteristics. They are undifferentiated cells that are self-renewing, clonogenic (form identical clones), and multipotent (able to differentiate into a wide array of specialized cell types). Stem cells can be categorized in a number of ways: anatomically, functionally, or by cell surface markers, transcription factors, and protein expression. The simplest and most common basic grouping of stem cells is based on their site of origin. Stem cells isolated from the embryo are named embryonic stem cells, and stem cells isolated from the adult are known as adult stem cells.

Embryonic stem cells

Embryonic stem cells (ESCs) are totipotent cells that possess the ability to differentiate into cells derived from the three germ layers: ectoderm, endoderm, and mesoderm. ESCs are derived from the inner cell mass of the blastocyst of a 3- to 5-day-old embryo. ESCs have been shown to be capable of generating functional cardiac, neuronal, and pancreatic cells in animal and human models. Boheler and colleagues [5] proved that ESCs are able to differentiate into cardiomyocytes represented by all specialized cell types of the heart, such as atrial-like, ventricular-like, sinus nodal-like, and Purkinje-like cells. These cardiomyocytes not only exhibit cell morphology similar to that of adult cardiac cells but also have similar physiology; cultured ESCs beat spontaneously, and when clustered, synchronously. Irrespective of their enormous regenerative potential, ESCs are enveloped in controversy related to their source of origin in humans and to their malignant degenerative potential. Therefore, to date, no clinical studies have been initiated in cardiac repair for humans.

Adult stem cells

Adult stem cells have less self-renewal ability than ESCs and the types of cells that can be created through transdifferentiation are limited. Adult stem cells reside in the postnatal bone marrow, blood, skeletal muscle, fatty tissue, and hearts of humans, making harvesting these cells for clinical application feasible.

Induced pluripotential stem cells Human induced pluripotential stem cells (iPSC) are derived from adult cells that have been reprogrammed back into an embryonic-like pluripotent state. Human induced pluripotential stem cells share more in common with fetal cells than with mature adult cells; principally, induced pluripotential stem cells beat irregularly and asynchronously. The fundamental goal of investigative work with human iPSC is to emulate the physiology and function of the adult myocardium. This requires persuading these induced pluripotential stem cells to differentiate and behave like mature adult cardiomyocytes resulting in synchronous regular beating.

At present, a precise understanding of how to coax and how to monitor for the successful coaxing of induced pluripotential stem cells into mature cardiomyocytes is incompletely understood. In addition, it is incompletely understood how to determine whether a human induced pluripotential stem cell-derived cardiomyocyte has achieved a sufficiently mature phenotype that might be suitable as a therapeutic modality.

Bone marrow cells: hematopoietic stem cells and nonhematopoietic mesenchymal stem cells Human bone marrow is composed of a cellular component and an extracellular matrix, with the latter containing cytokines and growth factors [6]. The cellular component of bone marrow is composed of differentiated cells, such as monocytes, lymphocytes, fibroblasts, adipocytes, chondroblasts, osteoblasts, and osteoclasts, as well as a fractionally small, but very diverse group of undifferentiated cells. The undifferentiated stem cell population is composed of hematopoietic stem cells (HSC), which include hemangioblasts and endothelial progenitor cells (EPCs), and nonhematopoietic mesenchymal precursor cells that give rise to stromal cells referred to as mesenchymal stem cells (MSCs).

The undifferentiated stem cell population can be isolated from differentiated cells by density gradient centrifugation. The end product of this centrifugation process is referred to as bone marrow mononuclear cells (BMMNCs), which contain the undifferentiated HSC and MSC as well as a few committed cells in various stages of maturation. The overall structure of BMMNCs is primarily that of early committed cells, with only 2 to 4% comprising HSC/EPC and approximately 0.01% of MSC [7]. This translates into approximately 2 to 5 MSCs per 1×10^6 BMMNCs [8, 9].

Undifferentiated BMMNCs (HSC and MSC) do not normally contribute to cardiac lineage cells. HSCs give rise to endothelial cells and all hematopoietic lineages. MSCs give rise to adipocytes, chondroblasts, and osteoblasts. MSCs are also human leukocyte antigen–antigen D related (HLA-DR) negative and, therefore, are believed to evade immune recognition [8, 9]. MSCs release cytokines and growth factors that can stimulate endogenous repair mechanisms [10]. MSCs under specific microenvironmental conditions can be induced in vitro to transdifferentiate into skeletal and cardiac muscle [8, 11, 12] and have been shown under uniquely precise conditions to transdifferentiate into cardiomyocytes in an in vivo

rodent animal model [13, 14]. Toma et al. [13] demonstrated the ability of human MSC to differentiate into cardiomyocytes when injected into the murine myocardium.

Although HSCs normally give rise to endothelial cells and all hematopoietic lineages [15, 16], under specific microenvironmental conditions, they have been shown in vitro and in select rodent animal models of injury to transdifferentiate into a wide variety of phenotypes including skeletal muscle [17], neurons [18], and hepatocytes [19]. It remains controversial whether these hematopoietic bone marrow lineage cells are truly able to transdifferentiate into cardiomyocytes.

Circulating EPCs In 1997, Asahara and collaborators [20] discovered that human peripheral blood contained a pool of cells capable of differentiation in vitro into endothelial cells. These circulating EPCs arise from the bone marrow and are a subset of BMMNC. EPCs retain the characteristics of stem cells, for they are self-renewing, clonogenic, and multipotent [21].

Different types of EPCs have been proposed, in particular "early" and "late" EPCs, based on the appearance in culture of these circulating mononuclear cells [22]. Early EPCs are believed to promote endothelial cell repair [23] and angiogenesis through paracrine effects, whereas late EPCs are believed to transdifferentiate into endothelial cells [24]. The differentiation of early EPCs into cardiomyocytes is controversial and not definitive [25]. Although the exact role for EPCs in cardiovascular physiology remains unclear, a number of animal studies indicate the protective role of EPCs in cardiovascular homeostasis. EPCs can be upregulated in the blood by the administration of granulocyte-colony stimulating factor (G-CSF). G-CSF has been shown to increase the number of EPCs in the circulation, permitting safe and efficacious mobilization and collection of EPC from the blood [26].

Cardiac stem cells Multipotent, clonogenic, and self-renewing cardiac stem cells (CSCs) exist within the myocardium and were first identified by Beltrami et al. [27] in 2003. These cells give rise to cardiomyocytes, smooth muscle cells, and endothelial cells in animal models of ischemia [28, 29]. CSCs are characterized by the stem cell antigen c-kit, cell surface antigens Sca-1, and MDR1 and do not express the hematopoetic surface antigens CD31, CD34, CD45, CD133, and KDR [29]. CSCs are distinct from progenitor cells that arise in the bone marrow and migrate to the myocardium. CSCs reside in myocardial niches within the heart where they divide and differentiate. CSCs participate in the normal turnover of cardiac cells by forming new cardiomyocytes and capillaries [30]. Despite its regenerative potential, the heart seems unable to defend itself adequately against ischemic or nonischemic injury. The reasons for this limited endogenous reparative ability remain unclear.

Davis et al. [31] and Milliaras [32] have described a related endogenous CSC, the "cardiosphere." Cardiosphere-derived cells (CDCs) are a naturally occurring mixed population of stem cells comprising endogenous CSCs (c-Kit+) and cardiac MSCs (CD90+ and CD105+), but not HSCs (CD45−). They are grown under very specific conditions. Cardiospheres are clonogenic and self-renewing and exhibit multilineage potential. Smith and colleagues [33] described the feasibility and safety of isolation and expansion of adult CSCs from human endomyocardial biopsy specimens.

Noteworthy clinical trials

To date, the majority of human stem cell clinical trials have involved cells of bone marrow origin. Bone marrow is readily available, and therefore, an accessible supply of multipotent cells. The total number of stem cells residing in the bone marrow of any human at any given time is insufficient for significant organ repair. Bone marrow aspiration, isolation, selection of a specific cell phenotype, and in vitro expansion may be required to achieve sufficient quantities of cells for successful therapy. MSCs could potentially serve as an allogeneic transplant, thereby avoiding the need for bone marrow harvesting from individual prospective recipients, an exciting therapeutic advantage. The commercial manufacture of these cells is now in progress and these cells are available for use in select clinical trials.

The many completed human clinical trials are noteworthy in that they have delivered either mixed or enriched bone marrow cells, administered a wide range of total cell numbers as well as different numbers of HSC and MSC cells, and infused cells at varying time intervals after myocardial injury. All of these inconsistencies have made comparing and interpreting the results of these trials rather challenging. Importantly, in all the trials in which the cells were harvested on the same day or within a few days of delivery, there is insufficient time to select, culture, and expand specific cell populations into large numbers for infusion. With a timetable of a few days, it is only possible to select for specific phenotypes that can be administered and only in small numbers. Therefore, in the majority of trials, especially those in which the bone marrow was harvested and delivered on the same day, the very small number of cells infused were a heterogeneous population that included HPCs, MSCs, and stromal cells. Trial authors may report the specific percentage of the infused cells containing specific stem cell phenotypic markers for HPCs (e.g., CD34+) or MSCs (e.g., CD105+), but one must recognize that these cells are present in infinitesimally small numbers compared with the total number of delivered cells.

The outcomes of the early trials can be summarized as having resulted in modest, inconsistent, and transient improvements in clinical endpoints. Notwithstanding these limitations, these first-generation trials have, nevertheless,

demonstrated the safety and feasibility of stem cell therapy and fueled the desire to pursue further clinical trials. Although some human studies have demonstrated mild improvement in LV function and rare attenuation of LV dilation (reverse remodeling), no study has to date documented true myocardial regeneration with the development of new cardiac myocytes or new blood vessels originating from the administered cells. Adding to the difficulty in the interpretation of results of these clinical trials is the fact that these trials have been conducted in patients with a wide range of ischemic heart disease ranging from acute infarction to chronic ischemic heart failure.

Cell therapy administration post myocardial infarction

TOPCARE-AMI Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) was one of the earliest clinical trials of stem cell therapy. Assmus and collaborators [34] randomized 20 patients in an open-label clinical trial to assess the safety, feasibility, and efficacy of unfractionated bone marrow-derived stem cells ($n = 9$) and circulating blood-derived EPCs ($n = 11$) injected into the infarct-related artery with a mean of 4.3 ± 1.5 days after an acute MI. The bone marrow-derived cells were harvested on the morning of infusion whereas the blood-derived EPCs were harvested approximately 3 to 4 days before planned infusion. Neither population of cells was a pure progenitor cell population. There was no difference in efficacy between the patients receiving bone marrow-derived stem cells or blood-derived EPCs. At 4-month follow-up, these cells were associated with a mild increase in global LVEF and minimally reduced end-systolic LV volume.

BOOST Wollert and colleagues [35] conducted a randomized control clinical trial on a similar but larger population of patients than in the TOPCARE-AMI trial. The BOne marrOw transfer to enhance ST-elevation infarct regeneration trial (BOOST) enlisted 60 patients, randomly assigned to receive either unfractionated BMMNCs ($n = 30$) or standard therapy with percutaneous coronary intervention ($n = 30$). BMMNCs were infused 4 to 8 days following PCI reperfusion for an acute ST-elevation MI. As in TOPCARE-AMI, a heterogeneous population of BMMNCs was injected into the infarct-related artery on the same day of harvest and 4 to 8 days after PCI reperfusion for an acute ST-elevation MI. After 6 months, mean global LVEF had increased by 7% in the BMMNC group and by 0.7% in the control group ($P = 0.0026$). By 18 months, the increase in LVEF was no longer seen [36].

ASTAMI Lunde and coworkers [37] in the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial noted no improvement in LVEF 6 months after delivery of a heterogeneous population of BMMNCs injected into the infarct-related artery 4 to 8 days after acute MI treated with PCI. The ASTAMI study randomly selected patients to receive PCI plus unfractionated BMMNCs ($n = 50$) or PCI alone ($n = 50$). These BMMNCs were harvested either on the day of or the day before intracoronary injection.

REPAIR-AMI The Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI), a multicenter, prospective, randomized, placebo-controlled trial treated 204 patients with acute MI. Patients received either autologous bone marrow cells or control medium injected into the infarct artery 3 to 6 days (mean 4.5 days) after PCI [38]. The bone marrow cell suspension consisted of a heterogeneous cell population that included hematopoietic, mesenchymal, and mononuclear cells harvested and infused on the same day. A statistically significant, but clinically irrelevant improvement in LVEF was observed in the cell-treated subjects at 4 months ($5.5\% \pm 7.3$ vs $3.0\% \pm 6.5\%$, $P = 0.01$).

The 2-year follow-up REPAIR-AMI data demonstrated marginally fewer MIs and fewer patients who met the combined endpoint of death, MI, or need for revascularization in the cell-treated patients [39]. The differences between groups in EF and LV end-systolic volume were not statistically significant or clinically relevant. This study provides the best long-term data to date regarding the safety and efficacy of bone marrow stem cell treatment in patients after an acute MI as well as the lack of clinical benefit from stem cell therapy with BMMNCs.

SCAMI Wöhrle and colleagues [40, 41] reported early and 3-year findings for the double-blinded, randomized, placebo-controlled intracoronary Stem Cell therapy in patients with Acute Myocardial Infarction (SCAMI) trial. Patients with acute MI were randomized to receive PCI plus BMMNCs or PCI plus placebo erythrocyte injection into the infarct-related artery 5 to 7 days after PCI. The cells were harvested and delivered on the same day and screened for hematopoietic stem cell markers. Forty-two patients were randomized in a 2:1 fashion (treatment:control). No differences were found between groups at 1, 3, and 6 months or 3 years for the primary endpoint of LVEF or in any of the secondary endpoints, which included major adverse coronary events, infarct size, and LV dimensions.

REGENT The Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) trial investigated intracoronary infusion into the infarct-related artery of a selected population of autologous bone marrow-derived CD34+ and CXCR4+ progenitor cells compared to a non-selected infusion of autologous BMMNCs in patients with acute ST

segment elevation MI and reduced LVEF successfully treated with PCI [42]. Bone marrow was prepared and infused on the day of aspiration and a mean of 7 days (3–12 days) after PCI treatment for an acute MI in patients with an LVEF less than 40%. Patients ($n = 200$) were randomly assigned to receive selected autologous ($n = 80$), unselected autologous BMMNCs ($n = 80$), or no cells ($n = 40$). At 6 months of follow-up, neither cell-treated group had an improvement in LVEF. There was a clinically insignificant absolute 3% increase in LVEF in both cell-treated groups, whereas the LVEF in controls remained unchanged.

Mesenchymal stem cells to treat acute myocardial infarction Hare and colleagues [43] investigated a unique preparation of allogeneic human bone marrow-derived mesenchymal cells (CD105+, CD166+, CD45−) in a double-blind, placebo-control, dose-escalation, multicenter phase I trial. All cells were isolated and expanded from a single donor and infused into the infarct-related artery anywhere from 1 to 10 days (mean 5 days) after thrombolytic reperfusion of an acute MI. Patients ($n = 53$) presenting with a first-time acute MI (ST or non-ST elevation) and an EF of 30% or higher were randomized in a 2:1 fashion (treatment:placebo) and treated patients were equally divided into three dose-escalation groups. After 3 and 6 months of follow-up, there was no improvement in LVEF with cell therapy. There was no difference in the incidence of adverse events between the placebo group and any of the three treatment groups, underscoring the safety of these allogeneic cells.

PreSERVE-AMI In the NBS10 Versus Placebo Post ST Segment Elevation Myocardial Infarction (PreSERVE-AMI) trial, ST-elevation MI patients received PCI stenting of the culprit lesion and were randomized to receive either autologous $CD34^+$ cells ($n = 78$) or placebo ($n = 81$) into the infarct-related vessel [44]. There were no differences between placebo and treated subjects for the primary safety endpoints of adverse events, serious adverse events, and major adverse cardiac events. No differences were observed in survival. The primary efficacy endpoint was not met, which was improvement in resting myocardial perfusion at 6 months. Furthermore, no changes in LVEF or scar size at 6 months were observed between groups.

MYSTAR The Myocardial Stem Cell Administration After Acute Myocardial Infarction study (MYSTAR) was a prospective randomized trial of BMMNCs delivered via both an intramyocardial (NOGA catheter, Biosense Webster, a Johnson & Johnson company, Diamond Bar, CA, USA) and an intracoronary route at either 3 to 6 weeks or 3 to 4 months after an ST-elevation MI in patients with LVEF less than 45% [45]. The cells were harvested and prepared the day before infusion. A total of 60 patients were treated. The combined dosing by two delivery methods and at an early and late time point resulted in a mild, but not clinically relevant decrease in infarct size and a marginal increase in LVEF at 3 months and 9 to 12 months after acute MI.

SWISS-AMI The Swiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction (SWISS-AMI) was a multicenter study of 200 patients with ST-elevation MI and LVEF < 45% who underwent PCI reperfusion within 24 h of symptom onset and received BMMNC (CD34+, CD45−, and CD133+) treatment into the infarct-related artery administered either "early" (5–7 days) or "late" (3–4 weeks) after acute MI [46]. The primary endpoint was a change from baseline to 4 months in global LVEF between the treatment and the control groups. No significant improvement in global LVEF was achieved with cell therapy whether or not the subjects were treated early (5–7 days) or late (3–4 weeks).

TIME trials The Timing in Myocardial Infarction Evaluation (TIME) trials were clinical trials exploring the delivery of BMMNCs at two distinct time points [47, 48]. The EARLY TIME trial [47] evaluated unselected BMMNC therapy 3 days versus 7 days post acute anterior wall ST-elevation MI and primary PCI in 120 patients with LVEF < 45%. BMMNCs were administered within 12 h of aspiration. The LATE TIME trial [48] evaluated BMMNC infusion at 2 to 3 weeks after acute anterior wall ST-elevation MI and primary PCI versus placebo in 87 patients with LVEF < 45%. BMMNCs were administered within 12 h of aspiration. No significant improvement in LVEF at 6 months was seen in either TIME trial, even in the subgroup with the most depressed LV function.

In the majority of BMMNC studies, the cells were infused early after MI and usually within days of an MI. Originally, this was considered the optimum time to administer cell therapy based on the anticipated presence of a reparative microenvironment induced by the MI. Growing evidence suggests that although the microenvironment elaborates cytokines, growth factors, and chemokines that promote cell homing and engraftment, it also elaborates inflammatory agents and proteins that destroy the transplanted cells. Comparing studies in which the cells were administered early (< 7 days) and late (> 2 weeks) after an acute MI, there is no difference between treatment groups and no clinical benefit with respect to improvement in LVEF, LV reverse remodeling, or sustained symptomatic improvement.

The explanation for these largely ineffective trials is multiple and includes the following: the technique for cell preparation is variable; the incubation period for selection and expansion varies; some cells are injected on the same day of harvest whereas others are delayed by a day or more; the exact nature of the injectate is unclear and most often a heterogeneous concoction of cells; the number and phenotype of the cells

injected are not always reported, and when reported, the total number of cells injected varies from study to study and often from patient to patient within a study; the technique of delivery is variable; the timing of delivery is inconsistent; the extent of LV dysfunction and geometry is variable; and the follow-up time period is often only weeks to months.

Cell therapy administration in chronic ischemic cardiomyopathy

As with clinical trials seeking benefit for patients after an acute MI, trials for chronic ischemic heart disease and LV dysfunction have met with mixed results. Perin and colleagues [49, 50] explored transendocardial administration of unfractionated BMMNC in patients with ischemic cardiomyopathy and a mean LVEF < 40%. There was no improvement in LVEF in the 11 treated subjects over control subjects at 6 and 12 months.

Hendrikx and associates [51] performed a randomized controlled clinical trial in 20 patients with postinfarction nonviable scar undergoing coronary artery bypass surgery who received direct intramyocardial BMMNC injections ($n = 10$) or saline placebo injections ($n = 10$) into the LV scar. The BMMNCs were harvested and prepared the day prior to planned infusion. At 4 months, there was no difference in global LVEF between treatment groups, but regional LV function in previously nonviable scar was mildly improved after BMMNC treatment.

TOPCARE-CHD The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study group examined cell therapy as a treatment for patients with ischemic cardiomyopathy who suffered an MI at least 3 months before enrollment in TOPCARE-CHD [52]. This unique controlled crossover strategy evaluated 75 patients with stable ischemic heart disease to receive either no cell infusion (23 patients), circulating endothelial progenitor cell (EPCs) infusion (24 patients), or BMMNC (28 patients) infusion into the coronary artery perfusing the most dyskinetic area of the left ventricle. Bone marrow aspiration was performed on the morning of cell infusion. On average, less than 1% of the BMMNC population was positive for the HPC marker CD34. At 3 months postinfusion, the patients in the control group were randomly assigned to receive EPC or BMMNC, and the patients who initially received BMMNC or EPC crossed over to receive EPC or BMMNC, respectively. At 3 months, intracoronary infusion of BMMNC, but not EPC or control, was associated with a mild, statistically significant, but clinically irrelevant increase in global LVEF (+ 2.9% increase in LVEF), which persisted after crossover, in other words, at 6 months after the initial infusion regardless of whether patients crossed over from control to BMMNC or from EPC to BMMNC. These results are somewhat different than the findings of TOPCARE-AMI in which both EPC and BMMNC infusion showed equal, but mild LVEF improvement. However, the measure of improvement was so small that the findings cannot be considered relevant to clinical practice.

Flores-Ramírez and colleagues [53] investigated the use of HPCS (CD133+) cells in seven patients with chronic ischemic heart failure. At the time of cell therapy, all seven patients were listed for heart transplantation and were not candidates for conventional therapies. Approximately 90 million cells were injected into the left anterior descending coronary artery in all patients. Evaluation 2 years posttreatment demonstrated that although ventricular volumes remained similar, LVEF as assessed by echocardiogram and MRI increased 10.8% and 10.2%, respectively ($P < 0.05$ for both). Furthermore, NYHA class improved in all patients. Although encouraging, these results must be balanced by the small sample size and the absence of a control group.

STAR The intracoronary Stem cell Transplantation in chronic heART failure (STAR) Heart Study [54] evaluated autologous BMMNC infusion into the infarct-related coronary artery in 191 patients with ischemic heart failure and an LVEF of ≤ 35% and a remote history of MI. The study was not blinded and patients who refused cell therapy served as controls and were followed prospectively. After 3 months, BMMNC-treated patients had significant improvements in their cardiac index, calculated LVEF (an increase of ~ 7%), NYHA class, and reductions in end-systolic (~ 15-mL reduction) and end-diastolic ventricular volumes (~ 10-mL reduction). These improvements persisted at 12 and 60 months posttreatment. No improvements were noted in the control patients.

The direct intramyocardial delivery of CD133+ BMMNC was evaluated in 20 patients undergoing CABG surgery and compared to 20 patients undergoing CABG surgery alone [55]. Patients had a mean LVEF ~ 37%. Bone marrow was harvested 1 day before surgery and isolated by magnetic separation with ferrite-conjugated antibody to select CD133+ HPC. Enriched BMMNCs were injected in the infarct border zone during the CABG operation. At 6 months, the LVEF rose from a mean 37.4 ± 8.4 to 47.1 ± 8.3% in the cell therapy plus CABG group ($P < 0.0001$), but only from 37.9 ± 10.3 to 41.3 ± 9.1% in the CABG-only group ($P < 0.012$). Although encouraging, these results must be balanced by the small sample size and broad standard deviation in LVEF.

POSEIDON-pilot The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis Pilot Study (POSEIDON-pilot) [56], a phase I/II randomized trial, compared autologous versus allogeneic MSCs to treat 30 patients with remote MI, chronic ischemic ventricular dysfunction, and an LVEF < 50%. Twenty million, 100 million, or 200 million cells (5 patients in each cell type per dose level) were

delivered by transendocardial catheter injection into 10 LV sites. At 1 year, neither allogeneic nor autologous MSCs improved LVEF. There were no ventricular arrhythmia or immunologic adverse events observed among allogeneic recipients.

MSCs in LVAD Ascheim and colleagues [57] conducted an NHLBI-sponsored Cardiothoracic Surgery Network clinical trial in patients with end-stage heart failure undergoing LVAD implantation. Thirty patients (2:1 randomization) received 25 million MSCs at the time of LVAD implantation. The primary efficacy endpoint at 90 days after randomization was functional status and ventricular function while temporarily weaned from LVAD support. There was no clear advantage to MSC administration, although MSC-treated patients experienced more weaning events and longer duration of weans. The allogeneic mesenchymal stem cells were immunoselected and expanded from a single bone marrow donor as a novel "off the shelf" product manufactured by Mesoblast. The stated advantage of MSCs is that they evade immune recognition because the cells lack HLA DR antigens.

CDCs: CADUCEUS and ALLSTAR Makkar and coinvestigators [58] evaluated CDC infusion in a prospective, randomized CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction human (CADUCEUS) clinical trial of patients with ischemic LV dysfunction (LVEF 25–45%) post-MI, successfully treated by PCI/stent. A total of 17 patients randomly received $12.5–25 \times 10^6$ autologous CDC grown from endomyocardial biopsy specimens infused into the infarct-related artery 90 days or less after MI. These CDC-treated patients were compared with 8 patients who were randomly assigned to receive PCI/stent. At 6 months, MRI analysis failed to show improvement in end-diastolic volume, end-systolic volume, or LVEF. However, statistically significant, but clinically irrelevant improvements with MSCs were seen in scar mass, in viable heart mass, and regional systolic wall thickening.

These authors followed this study with ALLogeneic Heart STem Cells to Achieve Myocardial Regeneration (ALLSTAR) trial, a phase I/II trial of an allogeneic, "off-the-shelf" cardiosphere-derived stem cell manufactured by Capricor [59]. Enrollment of 124 patients (randomized 2:1), 30 days to 1 year post-MI with LVEF ≤ 45% and infarct size ≥ 15% of LV mass received intracoronary CDC infusion into the infarct-related coronary artery. At 12 months, ALLSTAR failed to meet the primary endpoint of scar size reduction by MRI.

Summary

The last decade has witnessed the publication of a large number of clinical trials, primarily using BMMNCs as the injected cell, which often were produced by different techniques, delivered in different doses via multiple routes of administration, and into patients chiefly with acute MI, but also including patients with chronic ischemia as well as patients with ischemic and nonischemic heart failure. It is no wonder that the field has been viewed as confusing to interpret, producing conflicting information regarding mechanism of action, ideal cell type, cell dose, route and timing of delivery, clinical indications, and clinical effectiveness. Despite this perspective, much has been learned through these first-generation clinical trials. The considerable advances in our understanding include the following: (1) cell therapy is safe; (2) cell therapy has been mildly effective; (3) in humans, BMMNCs do not transdifferentiate into cardiomyocytes or new blood vessels; and (4) insufficient numbers of cells have been injected with poor early retention and poor engraftment.

The primary mechanism of action for cell therapy is now believed to be through paracrine effects that include the release of cytokines, chemokines, and growth factors that inhibit apoptosis and fibrosis, enhance contractility, and activate endogenous regenerative mechanisms through endogenous circulating or site-specific stem cells. The new direction for clinical trials includes the use of stem cells capable of cardiac lineage, such as endogenous CSC, priming with molecular homing signals, genetically engineering stem cells to express cardiac lineage or chemokines and growth factors, and the application of biomaterials to support a disrupted extracellular matrix. A promising area of stem cell therapy for cardiac repair is the use of enriched stem cell populations such as CSCs and CDCs. One should anticipate that the continued efforts to combine insights derived from animal studies and well-designed clinical trials will one day lead to stem cells becoming an effective part of the clinical armamentarium for the treatment of ischemic heart disease.

Compliance with ethical standards

Conflict of interest None.

Ethical statement This manuscript was written by me.

Animal and human statement Not applicable.

Consent form statement Not applicable.

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