

# Origin and Selection of Stem Cells for Cardiac Repair after Myocardial Infarction

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## Abstract

Despite state-of-the-art methods for the early diagnosis and treatment, heart failure resulting from myocardial infarction (MI) continues to be a major cause of morbidity and mortality worldwide. Most of the existing treatment modalities against heart failure are symptom-based, short-term and do not prolong survival. Stem cell-based therapy is promising strategy to lead to cardiac repair after MI. Over the last decade, stem cells with diverse origin, identity, and plasticity have been utilized for the regeneration and repair of damaged myocardium after MI, both in animal models and humans. The major challenges and dilemmas in stem cell therapy after MI included- ethical concerns and alloreactivity (with embryonic stem cells), malignant transformation and vector contamination (with inducible progenitor cells), coronary restenosis (with mobilization of bone marrow stem cells), and cardiac arrhythmias and structural heterogeneity due to non-coupling of cardiac and non-cardiac skeletal cells (with skeletal myoblasts). Therefore, as much as the progress made in the field of cardiac regenerative therapy, questions have been asked on what constitutes the most appropriate source for the stem cells. In particular, the identity, characteristics and ability of the stem cells to retain their fate while being propagated *ex vivo* have invited a passionate discussion among cell-biologists, geneticists and clinicians. This review summarizes the diverse origin of the stem cells and discusses recent advances made for the identification, selection and propagation of stem cells for the regeneration or repair of damaged myocardium after MI.

**Keywords:** Stem cells; Myocardial infarction; C-kit; Heart; Regeneration

## Introduction

Cardiovascular diseases (CVD) constitute the single leading cause of death in the United States. According to the heart disease and stroke statistics released by American Heart Association in 2010, 1 out of every 2.9 deaths is due to cardiovascular disease. Nearly 2600 Americans die of CVD each day, roughly one death every 34 seconds. Approximately every 25 seconds, an American will have a coronary event, and approximately every minute, someone will die of one. Even with early diagnosis and timely reperfusion of ischemic myocardium, up to 30% patients develop left ventricular (LV) remodeling and loss of cardiac function [1,2].

Current treatment modality of cardiac failure is mainly focused to relieve symptoms. The principle pathophysiologic mechanisms that are currently targeted are inhibition of the renin-angiotensin and aldosterone system,  $\beta$ -adrenoceptor antagonism, pre-load reduction with diuretics, and after-load reduction with nitrates and arterial dilators [3]. Placement of a Bi-ventricular automatic intracardiac defibrillator (BiV AICD) and left ventricular assist devices (LVAD) have opened a new showground for the management of heart failure [4-6]. However, preventing the progression of the disease and development of heart failure has remained a major challenge. Although cardiac transplantation has offered some hope, organ shortage, donor-recipient organ mismatch, and transplant rejection are the major challenges limiting its widespread use.

Unlike many other tissues in our body, adult cardiomyocytes have limited ability to self-regenerate. This means, an ischemic injury will lead to irreversible myocardial damage leading to loss of cardiac function and progressive cardiac dilatation or scarring. Stem cells have emerged as a promising strategy for cardiac replacement or repair after acute MI [7]. From a historical perspective, a brief insightful

review article titled- "Reparative Processes in Heart Muscle Following Myocardial Infarction" authored by RJ Bing in 1971 described the appearance of round cells in the border-zone of acute MI after the surge of acute inflammatory cells but stopped short of explaining or characterizing these cells [8]. Murry et al. [9] sought to redirect heart to form skeletal muscle instead of scar by transferring the myogenic determination gene, MyoD, into cardiac granulation tissue. Further studies in animal models of MI demonstrated that several subsets of adult primitive cells can regenerate cardiomyocyte with improvement in cardiac function. However, the last 10 years have witnessed an exponential increase in literature about the therapeutic use of stem cells after acute MI. A pubmed search using the keywords "Stem Cell(s) Myocardial Infarction" yields a total number of 2183 peer-reviewed articles. Over 300 peer-reviewed articles have already been published within the year 2010. Multiple clinical studies have examined the safety and efficacy of stem cell therapy after acute MI. Majority of the initial clinical trials, although diverse and heterogeneous in their design and execution, have shown that stem cell therapy is safe and leads to, at least, modest improvement of cardiac function.

Stem cells represent specific population of cells that are characterized by self-renewal (ability to undergo multiple cycles of cell division while maintaining its undifferentiated state) and clonogenicity

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(ability to spawn colonies of various differentiated somatic cell types). There is ongoing debate on what constitutes the best source of stem for the repair of damaged myocardium following an MI. To date, most of the studies have used autologous stem cells derived from bone marrow and peripheral blood. Studies that have used allogeneic human mesenchymal stem cells (hMSCs) following acute MI have, at least, established the safety profile of allogeneic stem cell therapy for clinical use [10]. Newly discovered stem cell types, e.g., resident cardiac stem cells and very small embryonic-like stem cells (VSEL-SCs) have been a focus of intense research to further characterize their plasticity, homing and growth characteristics, safety and efficacy to repair damaged myocardium and improve cardiac function. To place the findings of previous studies in context with emerging studies regarding the origin and selection of stem cells and its impact on the recovery of cardiac function, a report on the advances in the selection and delivery of stem cells is presented here.

### Selection and Differentiation of Stem Cells

Selection of stem cells for myocardial regeneration has become a subject of intense research. At the broader scale, stem cells can be divided into 2 major categories- embryonic and adult. However, with the advent of inducible pluripotent stem cell (iPSC), this division has become less distinct. A second and more common method of stem cell classification is based on their commitment to differentiate into adult tissues. The term “totipotency” refers to the ability of a single cell to differentiate into a complete organism (including extra-embryonic tissue). Embryonic stem cells (ESC) are derived from the inner cell mass of a blastocyst and do not contribute to the formation of cyto- or syncytio-trophoblasts. Therefore, ESCs are pluripotent (not totipotent) cells. Multipotent cells, in contrast, can give rise to multiple but limited numbers of lineages. Unipotent cells have the ability to differentiate into a single type of cell lineage.

### Pluripotent Stem Cells

Pluripotent cells are able to differentiate into all of the 3 primary germ layers (ectoderm, endoderm and mesoderm) and these cells are able to program themselves indefinitely.

### Cells obtained from the inner cell mass of an early embryo

Embryonic stem cells are derived from the inner cell mass of an embryo at an early stage of its development [11]. Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood. Because of their plasticity and self-renewal ability, pluripotent stem cells constitute promising tools for regenerative medicine [12]. However, this method of cell retrieval has ethical challenges, limited host availability and still faces the risk of HLA-mismatch, expression of pro-inflammatory cytokines and co-stimulatory molecules, and graft-versus-host disease.

### Inducible pluripotent stem cell (iPSC)

A viable alternative for germline-derived pluripotent stem cells was reported in 2006 by transfecting the pluripotency-related genes into non-pluripotent cells (adult fibroblasts). However, this iPSC line showed significant DNA methylation errors and failed to produce viable chimeras [13]. Since then, multiple studies have advanced the iPSC generation technology using several different pluripotency-related genes (Oct3/4, Sox2, Klf4, c-Myc, NANOG, LIN28 etc.) using either a retroviral or lentiviral system of gene delivery [14-16]. With the advent

of a novel “electroporation” technique, there is a possibility of new gene insertion into the cells without using a viral vector. The major concern for the clinical application of iPSCs is their tumorigenicity. Although iPSCs have cleared the major hurdle of ethical anxiety and immunological challenge of HLA-mismatch and graft-versus-host disease, further studies that address the oncogenic potential and malignant transformation of these cells are still in progress.

### Multipotent Stem Cells

#### Skeletal myoblasts (SM)

Skeletal muscle includes satellite cells, which reside beneath the muscle fiber basal lamina and mainly represent committed myogenic precursor cells and express the stem cell markers Sca-1 and CD34. Cardiac muscles are skeletal in nature and require an ability to tolerate ischemia in an event of decreased coronary perfusion and low output states [17]. SMs are committed to myogenic lineage, have high proliferative capacity, and can tolerate a prolonged ischemia. The high resistance to ischemia promotes their survival after injection into the target area, typically a post-infarct scar. Since these cells are autologous in origin, it avoids a major immunological challenge of HLA-mismatch, graft-versus-host disease and rapid clearance of donor cells by reticuloendothelial cells [18,19]. However, there are several concerns with the use of such cells post-MI. Because donor cells are not functionally coupled with the host myocardium; a direct contribution for synergistic contractility to improve myocardial ejection is unlikely. In addition, 2 important proteins, N-cadherin and connexin 43, involved in electromechanical coupling in myocardium are downregulated in SM-derived cardiomyocytes [20]. This can create a state of electrical heterogeneity leading to arrhythmogenicity. Clinical trials evaluating the role of SMs after acute MI are on progress, and further evidence on the safety and efficacy of these cells for clinical use is awaited.

#### Adult bone marrow-derived cells (BMC)

In 1986, a 12-year-old boy with acute lymphocytic leukemia received donor bone marrow from his histocompatible father whose marrow was harvested 40 minutes postmortem after he suffered a myocardial infarction. The marrow was stored in liquid nitrogen for 17 days prior to infusion into the recipient. The patient died of complications relating to graft-versus-host disease 67 days following transplantation [21]. This approach, although dismal at the outset, opened a new era to explore the possibility of treating Acute MI with adult bone marrow-derived stem cells. Bone marrow comprises several different cell types including hematopoietic stem cells, mesenchymal (or stromal) stem cells and progenitor cells. Two comparable studies published by Jackson et al. [22] and Orlic et al. [23], showed the formation of new cardiomyocytes into the damaged myocardium after the infusion of adult mouse bone marrow-derived stem cells. Since then several clinical trials have taken place with the use of CD133<sup>+</sup> or CD34<sup>+</sup> mononuclear cells, either via the mobilization of these cells with granulocyte colony-stimulating factor (G-CSF) therapy, or infusion of these cells through intracoronary or intramyocardial route [24-28]. The results have been mixed, but arguably there are several discrepancies on the design and methodology of these experiments.

Pre-cultured bone marrow-derived human mesenchymal stem cells (hMSCs) represent an alternative approach to cardiovascular cell therapy that has a number of advantages when compared with

autologous BMCs [29]. Because of their ability to home to areas of injured myocardium, hMSCs can be infused intravenously post-MI. These cells lack major histocompatibility complex and costimulatory cell-surface molecules, and can be used as allogeneic grafts. A small clinical study with intracoronary administration of autologous bone marrow mesenchymal stem cells showed a significant improvement of left ventricular function after acute MI [30]. A more recent double-blind, placebo-controlled study with intravenous infusion of allogeneic hMSCs has provided additional safety and efficacy data for utilizing these cells after acute MI [10].

VSEL-SCs are adult bone marrow-derived stem cells smaller than red blood cells but larger than platelets. These cells express a pan-stem cell marker Sca-1 in mice and CD133/CD34 in humans. These cells are not committed leukocyte (CD45<sup>+</sup>) or any other hematopoietic lineage (LIN<sup>-</sup>) [31]. However, these cells have the potential to differentiate into cardiomyocytes and endothelial cells. Although, preliminary studies in mice have shown a remarkable improvement of cardiac function following a transplantation of freshly isolated cells, major challenges still persist on whether an adequate number of cells can be retrieved or propagated without losing their identity or function [32].

The exact mechanism of action of bone marrow stem cells remains unclear. However, it has been recently proposed that the beneficial effect of bone marrow therapy can result from the activation of resident cardiac progenitor cells, by a paracrine mechanism [33,34]. Since stem cells and tumor cells have many common features, including self-renewal, multidrug resistance, and telomerase expression, there were significant concerns about the tumorigenesis following the mobilization of stem cells with growth factors. In addition, rapid restenosis of the coronary artery, possibly due to the differentiation of progenitor cells into smooth muscle cells within the stented segment, was reported in some studies [35].

### Resident cardiac stem cells (CSC)

The central dogma that the heart is a terminally differentially organ and has no capacity to regenerate has been challenged recently after the discovery of resident cardiac stem cells [36]. Recent studies in both animal models and humans have shown a population of c-kit (CD117)-positive cells in the myocardium that have the capacity to differentiate into cardiomyocytes. The CSCs are normally involved in maintaining myocardial cell homeostasis throughout life. These resident CSCs, through both cell transplantation and *in situ* activation, have shown the capacity to regenerate segments of myocardium, thus restoring anatomical integrity and ventricular function. The discovery of these cells has offered a new hope for the treatment of chronic myocardial ischemia, acute MI and chronic ischemic cardiomyopathy [37,38]. Interestingly, human atria have the higher density of these cells, thus enhancing the feasibility of cell-harvest from patients undergoing open-heart-surgery.

A randomized, open-labeled study with intracoronary injection of resident CSCs is currently in progress as a collaborative project between Brigham and Women's Hospital and our institution. This phase I clinical trial will involve 20 patients and 20 controls for an initial assessment of safety and feasibility of intracoronary cardiac stem cell therapy in humans. Patients with a history of Q-wave MI and LV ejection fraction (LVEF) < 40% who are scheduled for surgical revascularization within few days of the initial screening are enrolled to this study. Resident CSCs are harvested through right atrial appendage

by enzymatically dissociating the myocardial samples. These cells are then enriched with the addition of xenogenic antibodies, propagated *ex vivo*, tested for purity and antigenicity and infused back to the patient through the intracoronary route. Patients are followed up and monitored for adverse outcomes, death, sustained/symptomatic ventricular tachycardia, infection, bleeding, MI, stroke, peripheral embolism in the hospital after drug administration, in the first month after the injection of CSCs and serially afterwards [39].

### Unipotent Stem Cells

#### Endothelial progenitor cells (EPC)

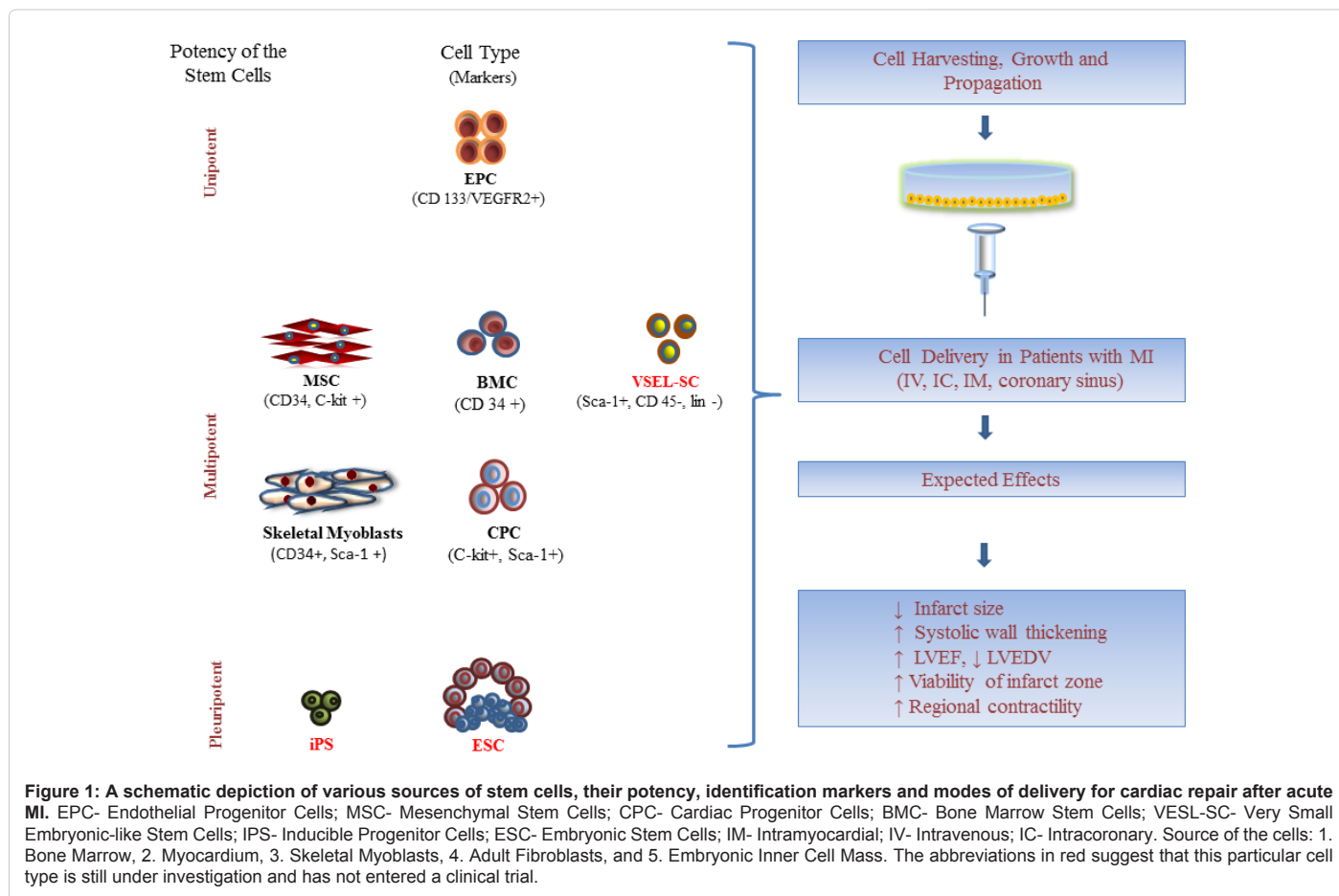
There are several other cell populations that have not been specifically tested in humans but have shown to improve myocardial function after acute MI. Asahara et al. [40] reported that purified CD34<sup>+</sup> hematopoietic progenitor cells from adults can differentiate *ex vivo* to an endothelial phenotype. The concept of EPCs has since been generated. Supporting the notion that these cells can regenerate endothelium, EPCs were also shown to populate Dacron grafts [41]. There are only limited numbers of studies that have examined the potential therapeutic role of EPCs for the treatment of acute MI as a potential treatment for various cardiovascular diseases. TOPCARE-AMI studied the therapeutic effect of EPCs that were either expanded *ex-vivo* from bone marrow or derived from peripheral blood culture. These studies showed significant improvements on ventricular ejection fraction, cardiac geometry, coronary blood flow reserve, and myocardial viability [42].

#### Other Cell Types

Other cells that have been investigated *in vitro* or in animal models include CD34<sup>+</sup> peripheral blood cells, omentum-based cell-supporting patch, administration of hMSCs encapsulated in RGD modified alginate microspheres, umbilical cord blood stem cells, and fibroblasts [43,44]. Indeed, a better understanding of their physiology, homing, engraftment and interaction with the vascular and myocardial milieu is needed before these cells can be considered for their potential clinical utility. Further information will be welcome mainly regarding the optimal cell type and cell-dose, pre-therapeutic mobilization and modification, antigenicity, and potential tumorigenicity of these cells, and more importantly, overall long-term safety of these cells in humans (Figure 1 and Table 1).

### Lessons from the Clinical Studies

Majority of clinical trials have primarily used autologous bone marrow-derived stem cells. These trials were performed predominantly in a male patient population of 18-75 years, within a week of suffering acute MI with a time scale for endpoint analysis ranging from one month to 18 months [28,30,47]. The improvement of cardiac function was reported to be modest in most of the studies. However, interpretation of these trials has met with the challenges because these studies differed widely on the source, dose and routes of stem cell delivery. Equally, a significant discrepancy existed in the timing of stem cell therapy, clinical-endpoints, treatment protocol for control subjects and methodology of data retrieval and outcome measurements. Out of 4 major randomized and controlled clinical trials that used G-CSF-dependent mobilization of mononuclear cells (MNCs), two studies reported increased systolic wall thickness following MNC-cell therapy, whereas only one study observed improvement of ejection fraction [24-26,51,52]. In contrast, most of the studies that have



Year	Event
1908	A Russian-American Scientist- Alexander A. Maximow proposed the term "stem cells". He stated that all the blood cells have a single precursor cell [45].
1996	Murry and associates sought to redirect heart to form skeletal muscle instead of scar by transferring the myogenic determination gene, MyoD, into cardiac granulation tissue.
2001	Shintani et al. [46] reported that lineage-committed endothelial progenitor cells and CD34+ mononuclear cells can be mobilized during an acute ischemic event in humans.
2002	Assamus et al. [42] reported that intracoronary infusion of autologous blood or bone-marrow progenitor cells is safe and feasible and may benefit post-MI remodeling.
2003	Stamm et al. [47] injected autologous CD133+ bone-marrow cells into the infarct border zone and suggested an improvement of myocardial perfusion is likely.
2003	Menasche et al. [48] reported that autologous skeletal myoblast transplantation for severe ischemic cardiomyopathy can improve regional contractility but might have arrhythmogenic potential.
2003	Beltrami et al. [37] reported multipotent resident cardiac stem cells that support myocardial regeneration.
2004	Kucia et al. [49] reported very small nonhematopoietic population of bone marrow-derived cells that express markers for cardiac differentiation.
2004	Kang et al. [35] injected G-CSF for the mobilization of PBSCs and administered these cells via intracoronary route to heart after MI. Although improvement of cardiac function was noted, a significant concern was raised for the possibility of coronary restenosis after stem cell therapy.
2009	Hare et al. [10] provided safety and provisional efficacy data for an allogeneic human mesenchymal stem cells in MI patients
2009	The first randomized and open-labeled phase I clinical study utilizing intracoronary injection of resident CSCs in patients with a history Q-wave MI and EF < 40% started recruiting patients [50].

**Table 1:** Landmark events in the history of stem cell discovery and its use for the therapy of myocardial ischemia.

utilized intracoronary approach to deliver CD34<sup>+</sup> or CD 133<sup>+</sup> cells have reported an improvement of cardiac function, decreased infarct size and increased viability of myocardial tissue [53-58]. Clinical trials with direct intramyocardial injection of stem cells, though fewer in number, have shown clear and unequivocal benefit for the improvement of LV ejection fraction and end-diastolic diameter [27,28,47]. Studies that have used hMSCs following acute MI have, at least, established the safety profile of allogenic stem cell therapy for clinical use [10].

It appears that with the emergence of new cell types and improved techniques for harvesting and delivering stem cells, we have moved much further ahead for a possible therapeutic use of stem cells to repair or regenerate damaged myocardium [50]. There are no definite studies to clearly explain the dynamics and homing profile of bone marrow-derived stem cells after acute MI. The biological interaction between the cellular grafts and host coronary vasculature as well as myocardium needs to be clearly investigated and interpreted. Novel genomic fingerprinting techniques offer a possibility of deciphering these interactions rapidly and at a large scale [59].

### Current Challenges and Future Directions

As much as the promises held for the improvement of cardiac function and healing of damaged myocardium, stem cell therapy has suffered a fate of controversy, ethical dilemma and methodological challenges. Myocardial regeneration after acute MI using stem cells is probably the most sophisticated and debated area germane to stem cell source, plasticity and mode of delivery. Use of pluripotent stem cells derived from the inner cell mass of Day-5 human embryos has faced serious ethical concerns. The iPSCs, initially considered being the “ultimate stem cells” for the regeneration of adult organs like heart, have sidestepped the major hurdle of ethical anxiety, HLA mismatch and immunological challenges related to embryonic stem cells. However, these cells have a potential for tumorigenesis and viral vector contamination. Mobilization of patient’s own hematopoietic stem cells with intravenous growth factor therapy was been challenged with increased incidence of coronary restenosis and possibility of tumorigenesis. Ongoing clinical trials utilizing resident autologous CPCs for treatment of ischemic cardiomyopathy have offered some hope and promise for the future of cardiac regeneration therapy, but the exact safety and efficacy profile of these cells is yet to be known. Other cell-types like umbilical cord blood stem cells, and fibroblasts are still in the early stage of investigation for feasibility of cell retrieval, characterization, propagation, and delivery.

The use of autologous stem cells can circumvent the need for complex immunological assays, high-dose and prolonged immune suppression of the host and possibility of acquiring graft-versus-host reaction following transplant. However, several studies have reported that use of allogeneic stem cells allow rapid generation of large number of cells from a small donor cell population [60]. Finding compatible donor cells to ensure engraftment of transplanted cells in an infarcted myocardium still remains to be a challenge. A “savior cell” from the immunocompatible siblings can intentionally be selected to match the immune status and harvest the cell type free of obvious inheritable disorder.

Several clinical trials using peripheral blood stem cells have been completed, and many others are currently underway. Two phase II trials (TRACIA and REGEN-AMI) are being conducted using autologous bone marrow stem cells [61,62]. With the identification of resident

cardiac stem cells and very small embryonic-like stem cells, we have challenged the traditional dogma that heart is a post-mitotic organ and have entered a most exciting and revolutionary part of stem cell biology and therapeutics. A phase I clinical trial is currently underway in our institution to evaluate the safety and efficacy profile of CPCs following acute MI [39]. However, more research is needed to clarify whether the stem cells with no genetic manipulation has the best potential for myocardial regeneration.

No consensus exists on what constitutes “the best cell type” for myocardial regeneration. But, arguably the most desirable cell would be multipotent, autologous, resistant to malignant transformation, and free of vector contamination. A solution for effective stem cell therapy for patients with acute MI depends on a successful liaison between molecular biologists, geneticists and clinicians. As much as it is important to identify the most appropriate source of stem cells that can regenerate myocardium after acute infarct, advances in the modality of cell harvesting, propagation, gene-incorporation, desensitization and cell tracking strategy are crucial determinants for adequate cell engraftment and naturalization, and most importantly enhancement of cardiac function.

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