

# Heart regeneration

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## Abstract

Heart failure is the leading cause of death worldwide and the main reason for hospitalization of patients 65-years of age and older. Current therapies only delay progression of the disease but the overall mortality remains as high as 20% per year. The underlying issue is the same whether the cause is acute damage, chronic stress from disease, or aging – progressive loss of functional cardiomyocytes and diminished hemodynamic output. Trials for regenerating cardiac muscle with cell-based therapy are ongoing for more than 10 years now. Multiple candidate cell types have been used in laboratory experiments and I generation clinical trials, including: skeletal myoblasts, bone marrow and peripheral blood mononuclear cells, endothelial progenitor cells, neonatal cardiomyocytes, mesenchymal stem cells, embryonic stem cells, induced pluripotent stem cells and cardiac stem cells. Results of these studies suggest that cell-based therapy for the failing heart can improve cardiac function. More recently, it has been shown that the heart is not terminally differentiated, and resident cardiac stem cells become a potential source for regeneration of cardiac muscle, smooth muscle and endothelium. This paper summarizes the results of I generation clinical trials and discuss prospects of regenerative therapy in the near future, especially in the context of new stem cell populations available.

**Key words:** heart regeneration, stem cells, pluripotent cells

## Introduction

Chronic heart failure is usually attributed to the loss of cardiomyocytes, with subsequent adverse remodelling of the cardiac muscle. Despite major therapeutic advances, heart failure continues to be a major cause of hospitalizations for patients above 65 years of age. Pharmacological treatment based on drugs with proven therapeutic benefits including  $\beta$ -blockers, ACE inhibitors and aldosterone antagonist diuretics has vastly increased the survival of patients suffering from heart failure. However, even the most perfectly tailored pharmacotherapy is not able to prevent mortality from heart disease which currently stands at 20% per year [1]. Advanced techniques for the treatment of heart failure, such as orthotopic heart transplant, mechanical left ventricular assist devices (LVAD) or artificial heart, are not commonly available and their application is limited to a small number of patients.

Consequently, there is an intense effort to find new better methods of treatment that would improve or possibly even completely restore the mechanical function of the heart, particularly among patients in advanced stages of heart disease who are not eligible for surgical treatment due to contraindications. During the last decade, the atten-

tion of scientists and physicians has been drawn towards the concept of cardiomyocyte renewal from stem cell populations. It is notable that no other area of medicine has recently been experiencing such rapid growth of regenerative therapy as cardiology.

Experimental animal studies and first-generation clinical trials conducted so far have investigated a variety of cell types including embryonic stem cells (ESC) [2], induced pluripotent stem cells (iPSC) [3], fetal cardiomyocytes [4], skeletal myoblasts (SKM) [5], bone marrow mononuclear cells (BMMNC) and peripheral blood mononuclear cells (PBMNC) [6], mesenchymal stem cells (MSC), endothelial progenitor cells (EPC) [7], and cardiac stem cells (CSC) [8]. The search for the perfect drug for patients with acute and chronic heart failure is ongoing and in the context of cellular therapy the main focus is on defining a population which:

- 1) is safe to the patient, i.e. carries no risk of cancer and does not increase the incidence of cardiac arrhythmia;
- 2) is effective, i.e. improves the mechanical performance of the heart by means of developing a new healthy cardiac muscle and blood vessels that are functionally integrated with the patient's tissues;

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- 3) is available as a standardized product and relatively inexpensive to administer;
- 4) can be applied in the clinical setting using minimally invasive methods;
- 5) is tolerated by the patient's immune system;
- 6) gives rise to no legal or ethical dilemmas.

The present article includes a summary of first-generation clinical trials and a discussion of prospects for the development of regenerative therapy in the foreseeable future.

## Endogenous mechanisms of cardiac regeneration

It has been known for quite a long time that the human heart has a limited regenerative potential compared to other tissues and organs such as the liver, skeletal muscles, intestine, bones or skin. The left cardiac ventricle has been shown to consist of ca. 2-4 billion cardiomyocytes [9]. Physiological ageing is associated with the process of natural death of cardiac muscle cells. A healthy heart is assumed to lose ca. 1 g of the myocardium (i.e. ca. 20 millions of cardiomyocytes) during 1 year [10]. Myocardial infarction, however, can destroy up to 25% of the cardiac muscle in the course of a few hours. Chronic cardiovascular diseases including arterial hypertension and valve defects of the heart result in a gradual but steadily escalating loss of cardiomyocytes [11]. The majority of cells in the adult cardiac muscle are mononuclear and polyploid [12]. They seem to preserve their proliferative capacity for the first couple of months of life. The final cycles of DNA synthesis usually do not involve a division of the nucleus and cytoplasm. The process leads to the formation of mononuclear cells with a multiplied DNA content ( $4n$  and more) [13]. Following the proliferation phase, cardiomyocytes enter the stage of physiological growth and increase their volume up to 30-40 times. In response to pathological stimuli such as arterial hypertension valvular heart disease, or following myocardial infarction, cardiomyocytes reinitiate DNA synthesis, however ultimately no divisions of the nucleus and cytoplasm take place [14]. Accordingly, the ploidy of cardiomyocytes increases, even up to  $64n$ . The process makes it significantly more difficult to interpret the results of examinations aimed at assessing the proliferative capacity of the human myocardium. Such assessments were published in the respected medical journal *Lancet* in 2009 [15].

In their experimental study, Bergmann *et al.* took advantage of the release of the radioactive isotope of carbon ( $^{14}\text{C}$ ) into the atmosphere during the Cold War period, followed by a rapid decline in  $^{14}\text{C}$  content after the entry into force of the Limited Nuclear Test Ban Treaty in 1963. Since  $^{14}\text{C}$  was absorbed into the digestive tract, it was possible to establish the age of cardiomyocytes by correlating  $^{14}\text{C}$  concentrations in the air and amounts incorporated into the cellular DNA. The renewal potential of cardiomy-

ocytes was found to be dependent on individual age. In humans at 20 years of age ca. 1% of cardiomyocytes are renewed per year, however in 75-year-olds the rate drops to 0.4%, which means that ca. 45% of heart muscle cells are renewed during a normal human life span. Even though this endogenous mechanism underlying cardiomyocyte regeneration is able to keep up with the physiological loss of cells taking place on an ongoing basis, it is not sufficiently effective to make up for the loss of cells arising, for example, during myocardial infarction.

## Skeletal myoblasts

Attempts to reinforce the regenerative potential of the heart have been undertaken for more than a decade. Repair activities focus on the one hand on the activation of endogenous mechanisms by supplying the damaged area with signals inducing proliferation and neoangiogenesis, and producing antiapoptotic and antioxidative effects. On the other hand, the goal is to increase the absolute number of cardiomyocyte progenitor cells in the risk zone.

The first cells to be used in the regenerative therapy of the heart were skeletal myoblasts due to their contractile properties, relatively high resistance to ischaemia and ease of accessibility for autologous procedures. In 2000, Menasche used skeletal myoblasts in patients with ischaemic heart disease undergoing surgical revascularization [16]. The follow-up period of several months revealed improved viability and contractility within the grafted tissue, confirmed with echocardiography and positron emission tomography (PET). At the same time, however, the study – as well as other research – showed an increase in the incidence of sustained ventricular arrhythmias, especially in the early postoperative phase [17]. Myoblasts are now known not to integrate electrically with the host tissues because their cellular membranes lack the protein connexin 43 which is a structural element of gap-junction intercellular communication. Consequently, grafted myoblasts hinder the transmission of active potentials in the muscle fibre and thus promote re-entry arrhythmia [18]. After grafting into the heart, skeletal myoblasts are transformed into multinucleated myotubes rather than cardiomyocytes.

Preclinical trials are currently under way to investigate populations of stem cells isolated from the blood vessels of skeletal muscle, the so-called myoendothelial cells, which are proposed to have a potential for differentiation into cardiomyocytes and endothelial cells. However, they do not express connexin 43 either, so complications in the form of cardiac arrhythmia disorders are to be expected [18].

## Bone marrow stem cells

Concurrently with Menasche's publication the first report was published on the application of bone marrow stem cells in the regenerative therapy of the heart [19].

The concept of using bone marrow emerged on the basis of extensive clinical experience with transplantation procedures, and the relatively easy access to significant amounts of autologous cells. Bone marrow is composed of at least several different populations of stem cells including haematopoietic stem cells (HSC) [20], MSC [21] and EPC [22]. Bone marrow mononuclear cells are the most common type of stem cells used for clinical applications. The population is very heterogeneous, consisting largely of cells representing all lines of the haematopoietic system at different stages of maturation. There are relatively few stem cells among them, with the proportion of HSC/EPC estimated at ca. 2-4% and MSC at ca. 0.01% [23]. The therapy uses both unselected BMMNC population and a subset of cells enriched with progenitor fractions. So far, they have been used mainly in patients after acute myocardial infarction and, to a lesser extent, in the treatment of resistant angina pectoris and chronic heart failure [24-26]. Unfortunately, haematopoietic cells have not been definitely proved to possess the capacity of transforming into cardiomyocytes *in vivo* [26]. Their effects are more commonly attributed to paracrine mechanisms (related to the secretion of cytokines and growth factors) and to the direct cytoprotective action (based on intercellular interactions and adhesive molecules) [9]. The analysis of available findings gives rise to a number of conclusions:

- 1) Intracoronary injection of bone marrow cells is a safe and relatively easy procedure.
- 2) The therapy produces a moderate clinical improvement, though study endpoints are not always achieved; furthermore doubts are raised over the sustainability of the therapeutic effect.
- 3) So far, the treatment has been practised predominantly among patients hospitalized due to the first myocardial infarction, treated by primary angioplasty, with a mild contraction deficit in the left ventricular muscle (EF ca. 50%), who fail to show significant improvement in response to therapy.

An interesting cell population among BMMNC are mesenchymal stem cells (stromal cells of bone marrow) [28]. They are unique in that they do not express of the major histocompatibility complex (MHC) class II molecules (HLA DR, DQ, DP) and adhesion molecules, which gives them low immunogenicity. As a result, they can be a source of cells for use in allogeneic therapy. This fact is all the more important because the process of ageing, just like advanced circulatory failure, significantly compromises the quality and the regeneration potential of haematopoietic cells. Consequently, the application of standardized allogeneic mesenchymal cells might be of value in terms of providing a more uniform therapeutic effect. Mesenchymal cells can be multiplied under *in vitro* conditions fairly easily. In addition, they are resistant to DNA-damaging factors and preserve the integrity of the genetic material in culture for up to several months. It is worthwhile to note

that the most spectacular effect of regeneration therapy reported to date concerns patients with acute myocardial infarction who received mesenchymal stem cells during angioplasty treatment. The patients had a 14% improvement in the ejection fraction of the left ventricle [29]. In view of their properties, mesenchymal stem cells seem an attractive target for research also from the viewpoint of the pharmaceutical industry.

### Cardiac stem cells

The search for natural progenitor cells for cardiomyocytes led, at the beginning of the present century, to the discovery and identification of CSC [30]. Cardiac stem cells are known to have the properties of self-renewal, clonal proliferation and differentiation into cardiac muscle, smooth muscle and endothelial cells. Cardiac stem cells are localized in the so-called cardiac niche, i.e. clusters of stem and stromal cells, which enables mutual associations and interactions with the extracellular matrix structures. Messina was the first to isolate CSC from fragments of the myocardium sourced from patients during cardiac surgery in 2004 [31]. Cardiac stem cells were sustained in culture in the form of spherical cellular conglomerates called cardiospheres (CSp). Despite encouraging results obtained in animal studies, cardiospheres have not found application in clinical trials because of fairly large molecule size (50-200  $\mu\text{m}$ ). Routine administration of molecules of this size through coronary vessels could turn out to be a risky procedure. Consequently, 3 years later Marban's team modified the isolation protocol and developed a method for acquiring a suspension of individual progenitor cells from cardiospheres (cardiosphere-derived cells – CDC) [32]. The starting material for isolation was tissue obtained from the patient by percutaneous endomyocardial biopsy. Cells cultured *in vitro* can be proliferated to obtain an appropriate amount (in the range of tens of millions) for clinical applications. The entire procedure takes ca. 4-6 weeks to perform despite a small amount of starting material. Cardiac stem cells are a morphologically heterogeneous population of cells, rich both in stem and stromal cells. No proarrhythmic effect or cancers associated with CDC administration have been noted in over 1,000 animal experiments conducted to date. More recently, the phase I/II clinical trial CADUCEUS (Cardiosphere-Derived autologous stem Cells to reverse ventricular dysfunction) was launched [33]. The trial involved patients after myocardial infarction, with left ventricular contractile dysfunction and the ejection fraction (EF) of ca. 25-45%. The patients received autologous cells 2-3 months after the ischaemic episode. One-year follow-up period showed reduced scarring after myocardial infarction as well as improved contractility and increased viable heart muscle mass compared to the control group receiving traditional treatment. Another phase I trial which is currently in progress (SCIPIO – Stem Cell Infusion in Patients with Ischaemic cardiomy-

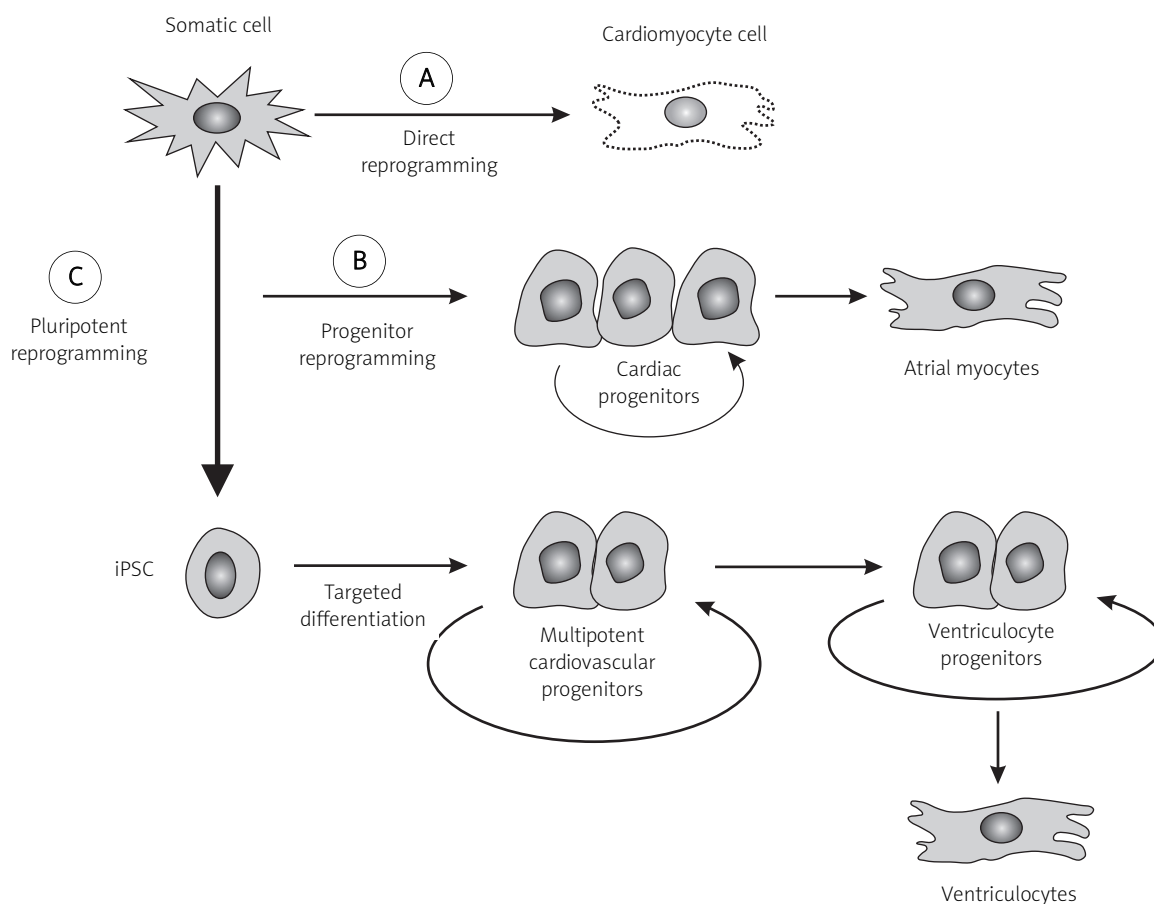
opathy) has already established the safety of intracoronary infusion of stem cells isolated from the heart [34].

### Pluripotent stem cells

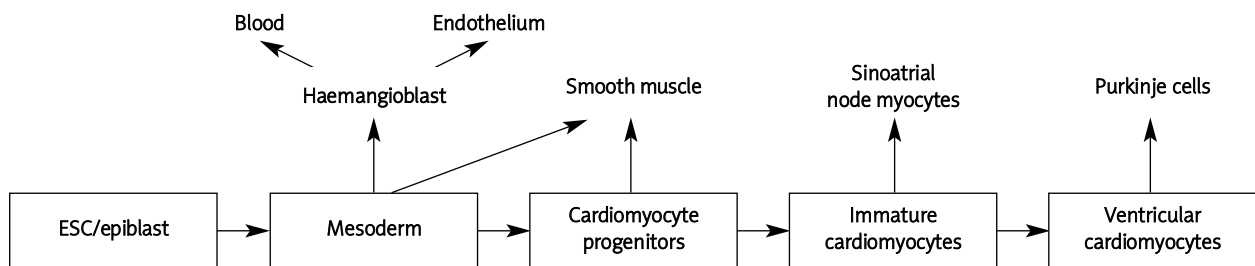
In general terms, stem cells can be described and divided on the basis of their renewal potential. Cardiac stem cells, similarly to HSC, are multipotent stem cells (i.e. have an ability to differentiate into one specialized group of cells, e.g. cardiomyocytes and endothelial cells or haematopoietic cells), whereas the greatest regeneration potential lies in pluripotent stem cells. These comprise ESC and iPSC. The latter originate from somatic cells (e.g. fibro-blasts, lymphocytes) which in the course of molecular manipulations acquire the morphological and functional properties of stem cells [35] (Fig. 1). Notably, authors of this discovery, Gurdon and Yamanaka, were awarded the Nobel Prize in Physiology or Medicine in 2012.

Embryonic stem cells, on the other hand, are derived from the inner cell mass of the embryo at the blastocyst stage [36]. Pluripotent stem cells have a capacity for unlimited proliferation and self-renewal; they can be differenti-

ated into tissues of the three germ layers: the ectoderm, endoderm and mesoderm, including cardiomyocytes [37] (Fig. 2). Pluripotent stem cells are relatively easy to culture *in vitro*. Since they divide efficiently, it is not difficult to obtain an appropriate amount for therapeutic purposes. Unfortunately, in view of a range of major obstacles, the cells have not found a prominent place in the clinical setting as yet [38]. Firstly, unlimited division capacity of undifferentiated cells carries the risk of cancer development. Usually, these are benign tumours called teratomas. Nevertheless, the risk of contaminating material with cells exhibiting malignant transformation potential cannot be ruled out. Secondly, the use of allogeneic cells entails the risk of rejection by the host's immune system. Consequently, immunosuppressive therapy would be necessary to maintain the effect of treatment. What is more, the sourcing of embryonic stem cells for therapeutic purposes is fraught with ethical and legal controversies [39]. An attempt to circumvent the issue was generation of induced pluripotent stem cells. For the time being, however, the efficiency of the process of reprogramming somatic cells is very low, ranging between 0.001% and 0.01% depend-



**Fig. 1.** Methods of sourcing cardiomyocytes from somatic cells: **A** – direct reprogramming into effector cells, **B** – indirect reprogramming into cardiomyocyte progenitor cells, **C** – indirect reprogramming into pluripotent stem cells



**Fig. 2.** Formation of effector lines of the cardiovascular system during the differentiation of embryonic stem cells and embryonic development

ing on cell type and on the initial cell number [40]. Further concerns are also raised regarding the methodology of cell preparation. Typically, genetic modifications are performed with viral vectors, whose integration within the genetic material may induce oncogenic transformation of cells. In order to find a way round this problem, works are currently under way to determine the suitability of episomal vectors, protein molecules and short-stranded RNAs [41]. The reprogramming of somatic cells takes ca. 4 months. A comprehensive assessment of the method must also take due account of high costs of therapy which must each time be customized to an individual patient [42].

Despite all the limitations, however, the regeneration potential of pluripotent cells is the main reason behind the scientific community's unwavering commitment to research focused on the application of the therapeutic capabilities of these cells in clinical practice. No resolution to these problems should be expected in the near future, though.

## Tasks for the future

First-generation clinical trials have not yielded unambiguous findings. While they have established without a doubt that cell therapy is possible and relatively safe in patients with advanced heart disease and using invasive methods, the trials conducted to date suffer from a number of weak points including:

1) Lack of uniform and standardized procedures governing cell preparation (unselected cell population vs. cells enriched with progenitor fractions, *in vitro* culturing), therapeutic doses (limited cell availability and their proliferative potential), routes of administration (direct – intravenous, intracoronary, epi- and endocardial injections; and indirect – mobilization of stem cells with growth factors), and the therapeutic window (administration during the coronary incident vs. in a remote period) [43].

2) Loss of cells between harvesting until implantation in the target tissue; estimates suggest that after 24 h, irrespective of the route of administration or cell type, the

**Table 1.** Characteristics of different populations of stem cells available for regenerative therapy of the heart (advantages and disadvantages)

Cell types	Advantages	Disadvantages	Application in clinical trials
ESC	<ul style="list-style-type: none"> <li>• Pluripotent</li> <li>• Unlimited potential for proliferation and differentiation</li> </ul>	<ul style="list-style-type: none"> <li>• Allogeneic</li> <li>• Uncontrolled potential for proliferation and differentiation</li> <li>• Legal and ethical dilemmas</li> </ul>	First clinical trials (neurology, ophthalmology)
iPSC	<ul style="list-style-type: none"> <li>• Autologous</li> <li>• Pluripotent</li> <li>• Unlimited potential for proliferation and differentiation</li> <li>• Easy to harvest</li> </ul>	<ul style="list-style-type: none"> <li>• Cost of therapy</li> <li>• Heterogeneous population</li> <li>• Uncontrolled potential for proliferation and differentiation</li> <li>• Modified genetic material</li> <li>• Potentially immunogenic</li> </ul>	No
BMMNC/MSC	<ul style="list-style-type: none"> <li>• Autologous</li> <li>• Possibility of using allogeneic cells of low immunogenicity</li> <li>• Easy to harvest</li> <li>• Extensive clinical experience</li> </ul>	<ul style="list-style-type: none"> <li>• Limited proliferation potential</li> <li>• Limited efficacy</li> <li>• Low cell viability</li> <li>• Uncertain cardiogenic potential</li> </ul>	Yes
CSC	<ul style="list-style-type: none"> <li>• Autologous</li> <li>• Proven cardiogenic potential</li> <li>• Mixed progenitor population</li> <li>• Rapid <i>in vitro</i> expansion</li> </ul>	<ul style="list-style-type: none"> <li>• Inadequate knowledge of cell biology</li> <li>• Non-standardized technique for <i>in vitro</i> culturing</li> <li>• Invasive harvesting methods</li> <li>• Intra-individual variability</li> <li>• Limited effectiveness</li> </ul>	First clinical trials

heart sustains less than 10% of the originally administered cells, of which further 90% die within a week; the process has been linked to cell washout via veins, mechanical ejection from the delivery site and apoptosis due to ischaemia, lack of contact with elements of the extracellular matrix and inflammation [44].

3) Uncertain cardiogenic potential of bone marrow stem cells; the formation of new blood vessels and the cardiac muscle occurs extremely rarely, if at all, in the circumstances; the disadvantages do not rule out the usefulness of these cell populations in regeneration therapy, e.g. in elderly patients, when the primary goal is not to reconstruct damaged cardiac muscle but to improve the quality of life; the considered mechanisms of action: through the production of cytokines and growth factors – cardioprotection, angiogenesis, inflammatory modulation, improved tissue metabolism; fusion of donor's and recipient's cells is also possible [45].

Another important issue concerns preclinical trials whose results have not translated into successful outcomes of regeneration therapy in humans. Based on the clinical experience gained so far, it is difficult to state with certainty which animal model is the best to use. Routinely, animals used for experiments are genetically uniform, young and healthy, not subjected to pharmacotherapy due to coexisting conditions and kept in strictly regulated environments. No natural animal model of ischaemic heart disease is known, either. Researchers customarily use mechanical closure of the coronary artery, which does not always reflect the essence of the clinical problem at hand [43].

While being aware of multiple imperfections associated in the method, one must not lose sight of the fact that stem cells are a powerful tool which, when properly applied, may turn out to be a major milestone in the treatment of some chronic illnesses. The discovery of CSC and efforts targeted at introducing pluripotent stem cells into the clinical setting are opening a new chapter in the regeneration therapy of the heart (see Table 1). However, further research in the field is necessary, with a special focus on large randomized clinical trials to clarify uncertainties, so that this form of treatment earns its permanent place in cardiac therapy.

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