Introduction

Prevention is the only chance to reduce morbidity and mortality from myocardial infarction (MI), and current therapies cannot restore the lost cardiac muscle tissue. In addition, human heart has a very limited regenerating potential, and common strategies aimed at preventing MI events are not always effective (1). Moreover, the only cure for patients with advanced heart failure (HF) is heart transplantation (2). Therefore, it is necessary to develop innovative therapies to repair the damaged cardiac muscle and improve the quality of patients’ life. This goal could be reached by applying cell therapy, the essence of regenerative medicine, to coronary heart disease (CHD). So far, several preclinical and clinical studies have been pursuing this strategy, which implies the transplantation of autologous stem or progenitor cells into the damaged myocardium in order to restore the lost function.
Unfortunately, clinical results are far from being satisfactory, because of the contrasting evidence about the benefits deriving from cell therapy in CHD. Indeed, even though some clinical trials report promising data, others do not show improvement in heart function after cell therapy. The only clear advantage reported in most of the clinical studies, is the reduction of the infarcted area and of non-functional scarring tissue. This review provides a critical update on the clinical studies evaluating the efficacy of cell therapy applied to CHD.

Cell sources

The final outcome of an ischemic damage to the myocardium is the formation of non-functional scar tissue. Therefore, modern approaches for MI are aimed at restoring the contractile function of infarcted myocardium. In particular, transplantation of stem and progenitor cells has been widely studied. Preclinical studies on small and large mammals have used different cell types, such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), skeletal myoblasts (Sk-Mbs), adult bone marrow-derived mononuclear cells (BM-MNCs), adult bone marrow-derived mesenchymal cells (BM-MSCs), endothelial progenitor cells (EPCs), adipose-derived stem cells (Ad-SCs), adipose-derived MSCs (Ad-MSCs), umbilical cord blood mesenchymal stem cells (UC-MSCs), cardiac stem cells (CSCs), and pericytes (1,3,4). Some of these studies proved that cell therapy can favor the repair of the damaged heart tissue (5-7). However, due to several concerns about the therapeutic use of certain types of stem or precursor cells, recent clinical trials mainly focused on bone marrow-derived cells, and in particular on BM-MNCs and on BM-MSCs (Tables 1,2). Especially, BM-MSCs have been the preferred source for cell therapy in MI.

ESCs are totipotent cells isolated from the inner mass of the blastocyst. These cells can differentiate into all the cell types of an organism, but safety and ethical issues limit their use in clinical practice (32). iPSCs are pluripotent cells derived from genetically reprogrammed adult differentiated cells. These cells can generate cell types from all three germ layers including cardiomyocytes (CMs) (33). The use of iPSC-derived CMs (iPSC-CMs) for clinical practice is encouraged by the good results obtained in preclinical studies. Nonetheless, many issues, such as a standardized protocol for iPSC-CM generation, safety, expensiveness of the procedures remain still unsolved and the time for this type of cells to be introduced into clinical trials has yet to come (33).

Sk-Mbs derive from satellite cells, the quiescent skeletal muscle precursors residing under the basal membrane of muscle fibres (34). Sk-Mbs have been transplanted in ischemic myocardium to repair damaged tissue, but, due to the limited evidence regarding their efficacy and safety, their use in clinical trials is very restricted (35).

BM-MNCs comprise hematopoietic stem and progenitor cells, mesenchymal stromal cells, embryonic-like stem cells, multipotent adult progenitor cells, hemangioblasts, EPCs and tissue-committed stem cells (36). As for the delivery strategies, intramyocardial and intracoronary injections seem to be the preferred routes.

Given the large spectrum of cell types included in the population of BM-MNCs, clinical trials have been performed on specific subpopulations of BM-MNCs, such as CD34+ and CD133+ cells. CD34+ and CD133+ are markers identifying also hematopoietic stem cells and endothelial progenitor cells which can differentiate into different types of adult blood and vascular cells.

BM-MSCs are multipotent cells that, under the appropriate stimuli, can differentiate into many tissues, such as bone, cartilage, fat, muscle, tendon.

EPCs are circulating cells derived from hematopoietic stem cells, that are capable of neovasculogenesis (37). These cells belong to the population of BM-derived cells and are characterized by specific surface markers such as CD31, CD34, and CD133 (38). Several studies demonstrated the potential benefits of EPCs in the treatment of MI, however very few are the clinical trials using selected EPCs for the treatment of patients with ischemic heart disease (39).

Adipose tissue contains mesenchymal stem cells (MSCs) and stem cells that can be used for cardiac repair strategies, as demonstrated in rodent models. In these studies, intramyocardial injection of adipose tissue-derived cells improved the recovery from MI (33). In addition, recent studies in humans have shown the beneficial effects of Ad-SCs transplantation in patients with ischemic cardiomyopathy (40).

As far as umbilical cord-blood cells, a heterogeneous population of cells including hematopoietic stem cells, MSCs, and somatic stem cells, their use in CVD is very limited, although these cells have high proliferative capacity and can give rise to different cell types, among which cardiomyocytes (41).

CSCs are multipotent stem cells residing in the heart with self-renewal and cardiac repair properties (42,43).
These cells can be isolated also by spheroid clusters, named cardiospheres (CSpCs), which derive from primary culture of myocardial biopsies (44). Recently, the effectiveness of these cardiosphere-derived cells in post-ischemic heart failure has been challenged in studies on rats (45).

Pericytes are cells of the microvascular wall, localised below the basal lamina underlying the endothelial cell layer of the microvessels. It has been demonstrated that these cells, in injured heart, can secrete different factors promoting angiogenesis, reducing fibrosis and apoptotic cell death in the damaged area (46-49). Moreover, intramyocardial injection of pericytes, together with cardiac stem cells, enhanced the repair of infarcted mouse hearts (50). Nevertheless, data on the beneficial effects of these cells on cardiac repair is limited, thus preventing their use in clinical trials.

Although there are many factors that limit the direct contribution of transplanted cells to myocardial repair process, such as cell engraftment, survival, proliferation, differentiation, and integration into the host tissue, benefits of this approach can last very long after the intervention, possibly because of the activation of endogenous repair process (1,51).

**Delivery routes**

In the clinical setting, intravenous, intracoronary, and intramyocardial injections are the three delivery strategies that have been used for cell transplantation. Each of them has advantages and drawbacks, thus an ultimate preferred procedure is not available yet. Nevertheless, in recent clinical trials, intracoronary and intramyocardial injections are the two most frequently used delivery routes for cell therapy (Table 1) (52).

Nowadays, in order to improve the benefits obtained with cell therapy, novel solutions are being investigated, such as those involving cell transplants supported by the simultaneous delivery of pro-repair drugs, synthetic scaffolds and growth factors (4).

**Recent published clinical trials**

Over the last 5 years, 20 clinical studies have been published on the use of cell therapy for CHD-derived conditions, such as acute myocardial infarction (AMI), CHD, left ventricular dysfunction (LVD), MI, angina (AG), HF, congestive heart failure (CHF) etc. (https://clinicaltrials.gov/). Among these studies, 13 (65%) gave positive results, whereas 7 (35%) gave negative results regarding functional recovery of the heart. Nevertheless, cell therapy led to a reduction of scar size, even in cases where there was no clear recovery of cardiac function in terms of ejection fraction. As previously stated, there is not a defined preference for a single delivery route, but almost all the studies used intracoronary or intramyocardial injections. Regarding the source of cells, there is a wide consensus on bone marrow cells (BMCs), due to the ease of BMC isolation and culture, as well as the presence of different multipotent stem cells in bone marrow (BM) (36).

In particular, clinical studies used either the heterogeneous population of BMCs, or different subpopulations of BMCs, such as BM-MNCs, BM-MSCs, BM-CD34+, BM-CD133+. The advantage of using the whole population of BMCs derives from its heterogeneity, given that it is still unknown what type of cell from the BM could be effective for cell therapy in CHD. On the other hand, the use of a very defined population of cells could be preferred to reach a standardized protocol.

The 13 clinical trials that yielded positive results are summarized below and reported in Table 1. In the NCT00962364, BMCs have been delivered by intracoronary injection in patients affected by chronic ischemic HF, resulting in an improvement of the cardiopulmonary exercise capacity. NCT00395811 trial showed that, at 12-month follow-up, the intramyocardial injection of BM-MNCs in patients with chronic MI during coronary artery bypass graft, led to a 5.5% improvement of the left ventricular ejection fraction (LVEF) and a significant improvement in contractility, without any variation in the infarct size. NCT00260338 trial demonstrated that intramyocardial delivery of BM-MSCs, in patients with CHD and refractory angina, improved exercise time, angina class, and weekly number of angina attacks. In NCT00474461, intracoronary infusion of autologous CSCs in patients with heart failure, after MI, improved left ventricular systolic function, reduced the infarct size and enhanced the viability of the myocardium. In NCT00587990, autologous MSCs have been injected intramyocardially in patients with chronic ischemic LVD secondary to MI, and undergoing coronary artery bypass grafting. As result, the treatment reduced the scar size and improved the perfusion and contractile properties of the injected myocardium areas. In NCT01291329 trial, 116 patients with acute ST-elevation MI (STEMI) were subjected to intracoronary injection of umbilical cord Wharton’s jelly MSCs. As result, the treatment led, at 18 months, to an increase in myocardial viability and perfusion in the ischemic area, to an increase in LVEF and...
a decrease in left ventricular end-systolic volumes (LVESV) and end-diastolic volumes. NCT00326989 trial showed that, in patients with HF, application of BMCs, after cardiac shock wave pretreatment, led to an increased LVEF, regional wall thickening, and reduction in the frequency of major adverse cardiac events. In NCT00644410 trial, intramyocardial injection of BM-MSCs in patients with congestive heart failure (CHF) caused a reduction in LVESV, and an improvement in LVEF, stroke volume, and myocardial mass. In NCT00810238 trial, BM-MSCs were differentiated into the cardiac lineage and injected intramyocardially in patients with chronic heart failure. As result, LVESV was reduced, and LVEF, 6-min walk distance, composite clinical score, physical performance, and event-free survival were improved. NCT01350310 trial demonstrated that transendocardial (TEC) administration of CD34+ cells in patients with ischemic cardiomyopathy led to an improvement in LVEF; an increase in 6-minute walk distance and a decrease in plasma concentration of the N-terminal pro B-type natriuretic peptide. In another trial with CD34+ cells (NCT01508910), intramyocardial injection of autologous cells in patients with refractory angina led to an improvement in total exercise time at 12 months and a reduced frequency of angina. In NCT02425358 trial, BM-MNCs were delivered into the coronary arteries of MI patients with ST-elevation, who underwent percutaneous coronary intervention. In the first 7 days following percutaneous coronary intervention, cell therapy increased LVEF, decreased LVESV, and improved myocardial perfusion. Intracoronary injection of BM-MNCs in MI patients enrolled in the clinical trial NCT01234181 showed improved improvement in left ventricular end-diastolic volume (LVEDV) and LVESV at 6 and 12 months. On the contrary, no enhancement was recorded in the LVEF.

In contrast with the previously cited clinical trials, seven published studies presented negative results for the use of cell therapy in ischemia-derived MI conditions: NCT00684021, NCT00418418, NCT00462774, NCT00984178, NCT00355186, NCT00765453, NCT00824005 (Table 1). These studies transplanted mainly BMCs either by intracoronary or intramyocardial delivery routes, and were applied mostly to patients affected by ischemic cardiomyopathies.

### Ongoing clinical trials

Interestingly, the ongoing clinical trials (Table 2), adopt the same delivery routes which have been used in the recently published studies, that is intramyocardial and intracoronary injections. On the contrary, cell type and sources that have been used in these more recent studies are clearly different from the past. In fact, many running clinical trials are based on the use of BMCs, but a significant percentage of studies (28%) utilizes different cell types and sources, such as cardiac stem cells, cardiac progenitor cells, adipose-derived stem cells and MSCs derived from umbilical cord blood. The clinical trial NCT01458405 is based on the injection of allogeneic cardiosphere-derived cells (CAP-1002) in the coronary artery of MI patients. This study is aimed at verifying the safety and effectiveness of the procedure, as measured by the size of the infarcted area. Intracoronary administration of allogeneic CSCs is the therapeutic strategy to treat patients with STEMI in NCT02439398 trial. Three clinical trials are based on the use of UC-MSCs: NCT02666391, NCT02323477 and NCT03180450. The study NCT02666391 is assessing the safety and efficacy of the intracoronary injection of UC-MSCs in patients with ischemic heart diseases; the clinical trial NCT02323477 is evaluating the efficacy of intramyocardial injection of UC-MSCs in patients with MI; in the clinical trial NCT03180450, allogeneic umbilical cord mesenchymal stem cells will be transplanted intravenously in patients with heart failure.

As for the use of AdSCs for the treatment of patients with LVEF ≤45% and heart failure, there are two clinical trials: NCT02673164 and NCT03092284. In these studies, the regenerative potential of intramyocardial injection of AdSCs will be evaluated. As already stated, the choice of cell type and source is of primary importance for the outcome of any cell-based strategy, as also highlighted by Cogle and collaborators, who studied the cellular composition of BM in patients with ischemic heart disease and severe LVD (53). Therefore, further knowledge is needed, but the enrichment of this research field with new factors and methods could push forward the use of cell therapy in CVD.

### Novel strategies for cardiac repair

Given the limited efficacy of current cell therapies for MI, several different approaches are being investigated (Figure 1). As an example, the group of Wai and collaborators has recently studied the role of follistatin-like-protein 1 (FSTL1) in the activation of vascular remodeling and cardiomyocyte proliferation (54). Based on the evidence that cardiac FSTL1 expression is lost upon MI, authors hypothesized that the recovery of the myocardial activity
of FSTL1 would activate cardiomyocyte proliferation, thus restoring the function of the damaged heart (54). Indeed, transplantation of collagen patches loaded with human FSTL1 to the epicardial surface of mouse infarcted hearts increased the formation of new vessels, the number of dividing cardiomyocytes and partially restored myocardial functions. This evidence highlights the potential of biomaterial-based strategies to treat patients affected by MI. In MI mice, the subcutaneous inguinal injection of tolerogenic dendritic cells (bone marrow-derived dendritic cells treated with tumor necrosis factor-α and cardiac lysate from MI mice) improved wound remodeling, left ventricular systolic function and survival (55). Very recently, the combination of genetic engineering with cell therapy has led to an improvement in the treatment of MI. Indeed, in rat models of MI, both the transplantation...
Table 2 Ongoing NIH clinical trials with a number of coronary heart disease patients >50

<table>
<thead>
<tr>
<th>NIH registration number</th>
<th>Status</th>
<th>No. of patients enrolled/to enroll</th>
<th>Cell type</th>
<th>Condition</th>
<th>Delivery route</th>
<th>Phase</th>
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<td>NCT00950274</td>
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<td>81</td>
<td>CD133+ cells</td>
<td>MI, CHD</td>
<td>IM</td>
<td>III</td>
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<td>100</td>
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<td>IC</td>
<td>II</td>
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<td>IC</td>
<td>I and II</td>
</tr>
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<td>AMI</td>
<td>BTC</td>
<td>III</td>
</tr>
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<td>II</td>
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<td>AMI</td>
<td>IC</td>
<td>II</td>
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<td>HF</td>
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<td>II</td>
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<tr>
<td>NCT01768702</td>
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<td>IC</td>
<td>II</td>
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<tr>
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<td>BM-MNCs</td>
<td>CA</td>
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<td>II and III</td>
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<tr>
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<td>CD34+ cells</td>
<td>HF, DC</td>
<td>IM</td>
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<td>NCT02323477</td>
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<td>BM-MNCs</td>
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<td>250</td>
<td>BMCs</td>
<td>HF</td>
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<td>III</td>
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<tr>
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</tr>
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<td>UC-MSCs</td>
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<td>I and II</td>
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<tr>
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<tr>
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<td>I</td>
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<td>NCT03092284</td>
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<td>AdSCs</td>
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<td>60</td>
<td>UC-MSCs</td>
<td>HF</td>
<td>IV</td>
<td>I</td>
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</table>

AdSCs, adipose tissue-derived stem cells; AG, angina; AMI, acute myocardial infarction; BMCS, bone marrow-derived cells; BM-MNCs, bone marrow-derived mononuclear cells; BM-MSCs, bone marrow-derived mesenchymal stem cells; BM-SCs, bone marrow-derived stem cells; BTC, balloon tipped catheter; CA, coronary atherosclerosis; CHD, coronary heart disease; CMI, chronic myocardial ischemia; CSCs, cardiac stem cells; CSpCs, cardiosphere-derived cells; DC, dilated cardiomyopathy; HA, heart attack; HD, heart disease; HF, heart failure; IDC, ischemic dilated cardiomyopathy; IHD, ischemic heart disease; IHF, ischemic heart failure; ICP, intracoronary; IOP, ischemic cardiomyopathy; IM, intramyocardial; IV, intravenous; MI, myocardial infarction; MPCs, mesenchymal precursor cells; NP, not provided; TEC, trans-endocardial; UC-MSCs, umbilical cord mesenchymal stem cell. Detailed information about the clinical trials are available on the web site http://clinicaltrials.gov/.
of eNOS-overexpressing BM-MSCs and HGF gene-engineered skeletal myoblasts significantly improved the repair and the functional recovery of the infarcted hearts (56,57). Interestingly, epigenetics has been clearly demonstrated to participate to the pro-fibrotic response of injured myocardium (58).

Several in vivo and in vitro studies demonstrated the role of miRNAs, in promoting cardiac muscle repair by stimulating cardiomyocyte proliferation and differentiation (59-61). In particular, miR-17-92, miR-23b, miR-199a, miR-204, miR-410, miR-495, miR-548c, miR-590, and miR-1825 can activate cardiomyocyte proliferation (59,60,62). Moreover, the inhibition of miRNA-23a and miRNA-92a expression reduced apoptotic death of rat cardiomyocytes after MI (63). Recent therapeutic approaches on MI explored also the use of exosomes to promote the repair of infarcted myocardium.

Exosomes are small endosomal membrane-bound vesicles secreted by cells for exchanging proteins, mRNAs and miRNAs. As reported in preclinical studies, exosomes from cardiac cells, MSCs and stem cells can be effective in the functional recovery of cardiac tissue from MI (64-67). Another very promising approach is based on the delivery of pro-repair factors, such as neuregulin, into infarcted hearts using biodegradable microparticles (68,69). These microparticles can escape the immune system and constantly release the active compound for 12 weeks, until they degrade. Additionally, a very interesting research led to the development of a spraying system for a mini-invasive application of platelet fibrin gel on the surface of infarcted mouse hearts. The layered polymerizable biomaterial patch attenuated LV remodeling, reduced cardiomyocyte death and stimulated myocardial repair, thus ameliorating heart function (70). However, powerful methods to monitor the effect of cell therapy are also needed (71).

**Conclusions**

The clinical use of cell therapy in CHD has not reached a mature stage yet, where defined and standardized procedures are available. A consensus on cell types, number of cells to implant, delivery routes, transplantation timing is still lacking, and the number of variables to account for is huge. Moreover, despite the increasing amount of data resulting from all the preclinical and clinical studies realized so far, it is difficult to compare different studies and to extract conclusive information from them, because of the differences in the strategies adopted in the studies.
Nevertheless, an important point to underline is that autologous cell therapy is safe, and successful results have been collected anyway by different groups. The potential benefits of an effective cell therapy in CHD are so important that even if its efficacy has been, so far, variable and limited, it is still very worthwhile to invest in this field.

**Acknowledgements**

None.

**Footnote**

Conflicts of Interest: The authors have no conflicts of interest to declare.

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