Transplantation of progenitor cells and regeneration of damaged myocardium: more facts or doubts? Insights from experimental and clinical studies
Massimo F. Piepoli

The development of postinfarction left ventricular dysfunction, particularly in patients with a large myocardial infarction, remains a major challenge. Recent experimental studies, showing that bone marrow cells may repair cardiac tissue, have offered renewed hopes for the prevention of this ominous occurrence. This optimism is further supported by encouraging results from some clinical trials in both acute myocardial infarction and in chronic cardiomyopathy, although the degree of benefits, the underlying mechanisms, and the cell types involved remain to be elucidated. This review summarizes the most relevant experimental experiences and all clinical studies supporting but also questioning the use of bone marrow cells in myocardial repair.

Introduction
The regeneration or replacement of functional tissues after heart damage has been traditionally considered a ‘mission impossible’ in cardiology. Reperfusion of the ischaemic myocardium was the only intervention available to restore the various cellular functions affected by myocardial ischaemia. Recently myocardium self repair by regeneration from autologous and undifferentiated primitive cells or differentiated cells with proliferative properties has been theorized [1]. However, this reparative process has demonstrated limited clinical importance due to the poor capacity of regeneration and proliferation of autologous human cardiomyocytes to prevent either the scar formation that follows myocardial infarction and the loss of heart function occurring in patients with cardiomyopathy and heart failure.

Recent hopes have risen from experiences that demonstrated the possibility of replacement and regeneration of functional cardiac muscle achieved either by stem cells.

The term ‘stem cell’ arose from the concept that these cells have properties analogous to those of the stem of a plant. In plants, the stem may grow to produce more stem, that is, more of itself, or different structures such as leaves or flowers. This elegantly illustrates the two key properties that define stem cells. Firstly, they have the ability to renew themselves for long periods through cell division. Secondly, under specific conditions they can differentiate into a spectrum of different cell types.

Stem cells have a hierarchy in terms of their ability to differentiate into other cell types. This ability is termed their differentiation ‘potential’. In nature, the stem cell with the greatest ability to differentiate into various different cell types is the zygote, which is termed ‘totipotent’ as all cell types of the body arise from it. An embryonic stem cell, which arises from subsequent division of a zygote, is termed ‘pluripotent’ as it is capable of differentiating into any cell type from any of the three germ layers (i.e., endoderm, mesoderm, ectoderm). Adult stem cells, which are present in all adult mammals, are termed ‘multipotent’ given their ability to differentiate into different tissue types. Finally, the committed progenitor cell is termed ‘unipotent’ as it is destined only to become one cell type.

Broadly, stem cells can be initially classified into embryonic or nonembryonic with the nonembryonic category being subsequently split into adult stem cells and cord blood stem cells. One of the central and recurring questions of all stem cell treatment modalities is which type of stem cells to use in which setting?

For heart regeneration different approaches have been proposed, such as the transplantation of allogenic cells (e.g. embryonic stem cells, bone marrow mesenchymal cells or skeletal myoblast) or by stimulation of autologous stem cells and/or resident progenitor cells.

Embryonic stem cells
The most primitive of all stem cells are the embryonic stem cells that develop as the inner cell mass in the
human blastocyst at day 5 after fertilization: embryonic stem cells have vast developmental potential as they can give rise to cells of the three embryonic germ layers. They can undergo cell proliferation and form embryo-like aggregates (termed embryoid bodies) in vitro, some of which can spontaneously contract. The beating embryoid bodies contain a mixed population of newly differentiated cell types including cardiomyocytes. Embryonic stem cells spontaneously differentiate into endothelial progenitor cells (EPC), haemangioblasts, mesenchymal stem cells (MSC). Haemangioblasts further differentiate generating both haematopoietic stem cells (HSC) and EPC, which give rise to both vascular blood and myocyte components. Embryonic stem cells have raised particular interest, since as the prototypical stem cell, they unequivocally fulfill all of the criteria of stemness: clonality, self-renewal, and multipotency [2,3]. Embryonic stem cells can differentiate into all cell types required in the adult and hold the potential to completely regenerate the myocardium. Ethical issues that ensued from human embryonic stem cell derivation requiring the destruction of human embryos, technical hurdles with maintaining survival of transplanted cells, and the concerns of immunologic rejection and teratoma formation stand as obstacles in the path of embryonic stem cell-based therapy [4]. However, these limitations may be overcome with advances in our understanding of the specification and differentiation of embryonic stem cell-derived cardiomyocytes, alongside methods to limit tumourigenesis, including genetic preprogramming or in-vitro differentiation before injection. One alternative was proposed by Takahashi and Yamanaka: [5] in mouse embryonic or adult fibroblast cultures could generate pluripotent stem-cell-like cells (induced pluripotent stem (iPS) cells), showing the essential characteristics of embryonic stem cells in terms of morphology, cell-surface markers, gene-expression profiles and telomerase activity. Furthermore, iPS cell clones could be maintained in culture for several months at least and could be induced to differentiate into derivatives of all three embryonic germ layers both in vitro and in vivo. These studies open up exciting prospects. However, the present methods for generating iPS cells require genetic integration by retroviruses or lentiviruses: [6] ongoing work focuses on influencing cardiomyocyte specification from embryonic stem cells by manipulating signalling to enable the therapeutic strategy of preselecting committed embryonic stem derived cardiac progenitors for transplantation [7]. In conclusion embryonic stem cells cannot be considered a viable option for cellular cardiomyoplasty until the several open issue have not been successfully solved.

Although different types of cells have been studied, in clinical settings adult skeletal myoblast cells and bone marrow stem cells have been more extensively evaluated, together with stimulation of stem and progenitor cells because their use bypasses much of the ethical and legal issues raised by the use of embryonic stem cells.

**Adult skeletal myoblast cells**

The first clinical studies of myocardial regeneration were performed using adult skeletal myoblast cells. When transplanted, these stem cells can successfully home and engraft within a damaged myocardium, preventing progressive ventricular dilation and improving cardiac function [8,9]. The myoblasts can be delivered into the myocardium by either intramural implantation or arterial delivery [10,11]. Skeletal muscle satellite cells can proliferate abundantly in culture; and can be easily grown from the patients themselves (autologous) thereby, avoiding potential immune response. Myoblasts are relatively ischaemia-resistant (compared with cardiomyocytes which become injured within 20 min) and they can withstand several hours of severe ischaemia without becoming irreversibly injured [12]. In animal model of dilated cardiomyopathy the use of skeletal myoblasts, delivered by multiple intramyocardial injections, was effective in restoring left ventricular function, demonstrating that the functional benefits of transplanted skeletal myoblast can be extended to nonischaemic cardiomyopathy [13].

**Human studies**

After an initial case report [14], few small, nonrandomized trials investigating the safety and feasibility of
Bone marrow contains several stem cell populations, including endothelial cells with overlapping phenotypes, including endothelial progenitor cells. The beneficial effects of BMC were shown on myocardial infarction in mice [21] in which implanted BMC could differentiate into myocytes and coronary vessels and similar findings of cell-mediated repair of myocardial infarction were reproduced in mouse model [27].

Clinical studies using skeletal myoblasts-based cell therapy in humans (with chronic ischaemic heart failure)

<table>
<thead>
<tr>
<th>Authors, (year)</th>
<th>Patients (n)</th>
<th>Design</th>
<th>Way and time of delivery after MI</th>
<th>Dosage of delivery (cells/ml)</th>
<th>Follow-up (months)</th>
<th>Baseline LVEF (%)</th>
<th>Changes in LVEF (unit%)</th>
<th>Other Major Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menache' (2003) [15,16]</td>
<td>10 treated</td>
<td>Non-RCT</td>
<td>TEp during CABG; 3-228 months</td>
<td>8.7 ± 1.9 × 10⁸</td>
<td>52</td>
<td>24 ± 4</td>
<td>* (p 4)</td>
<td>Regional wall motion</td>
</tr>
<tr>
<td>Smits (2003) [17]</td>
<td>5 treated</td>
<td>Non-RCT</td>
<td>TEn; 24-132 months</td>
<td>1.9 ± 1.1 × 10⁴</td>
<td>6</td>
<td>36 ± 11</td>
<td>* (p 9)</td>
<td>Regional wall motion</td>
</tr>
<tr>
<td>Siminiak (2004) [18]</td>
<td>10 treated</td>
<td>Non-RCT</td>
<td>TEp during CABG; 4-108 months</td>
<td>4-5 ± 10⁹</td>
<td>12</td>
<td>35.2</td>
<td>* (p 6.8)</td>
<td>Regional wall motion</td>
</tr>
<tr>
<td>Chacques (2004) [19]</td>
<td>20 treated</td>
<td>Non-RCT</td>
<td>TEp during CABG; NA</td>
<td>3.0 ± 1.2 × 10⁸</td>
<td>14</td>
<td>28 ± 3</td>
<td>* (p 24)</td>
<td>Regional wall motion</td>
</tr>
<tr>
<td>Dib (2005) [20]</td>
<td>30 treated</td>
<td>Non-RCT</td>
<td>TEp during CABG or LVAD; NA</td>
<td>3 ± 10⁹</td>
<td>24</td>
<td>28</td>
<td>* (p 8)</td>
<td>Regional wall motion and viability</td>
</tr>
<tr>
<td>Gavira (2005) [21]</td>
<td>12 treated</td>
<td>Non-RCT</td>
<td>TEp during CABG; 3-166 months</td>
<td>1.9 ± 1.2 × 10⁴</td>
<td>12</td>
<td>36 ± 8</td>
<td>* (p 20)</td>
<td>Regional wall motion and viability</td>
</tr>
<tr>
<td>Causmic (2008) [22]</td>
<td>12 treated 11 controls</td>
<td>RCT</td>
<td>TEn; 24-132 months</td>
<td>3-60 ± 10⁹</td>
<td>12</td>
<td>&lt; 40</td>
<td>*</td>
<td>Regional wall motion and viability</td>
</tr>
<tr>
<td>MAGIC (2008) [23]</td>
<td>97 treated 30 controls</td>
<td>RCT</td>
<td>TEp during CABG &gt;4 weeks</td>
<td>4-8 ± 10⁹</td>
<td>6</td>
<td>25-28</td>
<td>¼</td>
<td>LVESV and LVEDV</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; CD133+ bone marrow-derived CD133+ cells; CPC, circulating blood-derived progenitor cells; IC, intracoronary; IM, intramyocardial; LVAD, left ventricular assist device implantation; MNC, bone marrow-derived mononuclear cells; MSC, bone marrow-derived mesenchymal cells; NA, not available; NC, bone marrow-derived nucleated cells; RCT, randomised controlled trial; RPCT, randomised placebo controlled trial; TEn, transendocardial; TEp transepicardial; year, year of publication.
stem/precursor cells (EPCs), haematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and multipotent adult progenitor cells (MAPCs) [28]. In experimental setting adult EPCs transdifferentiate into active cardiomyocytes [29], although how extensively this occurs in clinical setting is presently unknown. Although this cell-mediated myocardial repair was initially characterized as resulting from HSC’s ability to transdifferentiate to cardiomyocytes, HSC plasticity has been difficult to reproduce and both its significance and basis remain undetermined.

Bone marrow-derived MSC and stromal cells (thought to have many properties of MSC) exhibit a high degree of plasticity allowing them to be employed as a self-renewing autologous source of progenitor cells (from adults), with the potential for differentiating into cardiomyocytes and can be used in cellular cardiomyoplasty in vitro and in vivo by creating three-dimensional aggregates up to 50% of human MSC express cardiac protein [30].

Upon treatment with specific agents (e.g. 5-azacytidine), MSCs can differentiate into synchronously beating cardiomyocytes [31]. The injection of MSCs after their expansion in culture can also be used in the rescue of an abnormal mouse cardiac phenotype [32] and may prove effective in repairing a broader array of cardiac damage including myocardial infarct. Furthermore, these bone-marrow derived stem/precursor cells also can prevent the progression of cardiomyocyte apoptosis and stem cardiac remodelling [33]. However, an experimental study using MSC subpopulation reported the appearance of microinfarctions following intracoronary delivery to a canine heart and suggested that care must be exercised when purifed BMC subpopulation are studied [34].

Furthermore the mechanism of BMC-mediated augmentation of cardiomyocyte number and function remains controversial: transdifferentiation [28], cell fusion with preexisting cardiomyocytes, paracrine effects of transfected cells have been hypothesized [35] Cell fusion has been demonstrated between cardiomyocytes and noncardiomyocytes in vivo and in vitro [36] whereas the data in support of transdifferentiation (particularly with HSCs) have not always been replicable. Further research is needed to clarify these issues.

Human studies in acute myocardial infarction
Inspired by the exciting experimental data, several trials were initiated to test whether cell therapy is well tolerated and feasible in patients after acute myocardial infarction (AMI). Some have decried the clinical trials as being premature without a more complete understanding of the underlying mechanisms [37], whereas others have pointed out that the clinical trials are justified by the potential benefits of cell therapy [38] All clinical studies included patients with AMI who had undergone primary angioplasty and stent implantation to reopen the infarct-related artery, and cells were infused intracoronary by using the stop-flow balloon catheter approach. In this regard, the clinical studies differ significantly from the animal studies, where the infarct related artery was not reperfused and cells were directly injected into the myocardium. The clinical trials using BMC are presented in Table 2 [22,39–51]: the combined experience from several hundreds of patients suggests that intracoronary delivery of unselected BMCs (all nucleated cells or mononuclear cell fraction only) is well tolerated in the short-term and mid-term (3–18 months). Furthermore in the larger study, the REPAIR-AMI trial, intracoronary infusion of BMC was associated with a reduction in the pre-specified combined clinical end point of death, recurrence of myocardial infarction, and any revascularization procedure at 1 year [49].

This trial has also shown that the patients with a baseline LVEF at or below the median value derived the most benefit. The magnitude of LV contractile recovery was inversely related to the baseline LVEF, confirming similar observations in the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) pilot trial [41]. Thus, enhanced recovery of contractile function may be beneficial specifically in patients with large infarcts and depressed LV function. Interesting a dose-related effect of autologous BMC transplantation on the myocardial function has been confirmed by a recent meta-analysis [52].

Clinical surveillance, Holter monitoring, and data from an electrophysiological study indicate that intracoronary BMC transfer is not associated with an increased propensity to ventricular (or supraventricular) arrhythmias. Direct injection of filtered nucleated BMCs into the acutely infarcted myocardium in rats has been found to induce intramyocardial calcifications. No evidence for intramyocardial calcifications (or tumour formation) has been obtained in patients in the longer follow-up study after intracoronary delivery of Ficoll or gelatin gradient-purified BMCs [47].

No bleeding complications were noted after bone marrow harvest. Intracoronary BMC infusions did not appear to inflict additional ischaemia damage to the myocardium or to promote a systemic inflammatory reaction, because no further increases in serum troponin or CRP levels were observed. No increased rates of n-stent restenosis were observed after transfer of unselected BMCs, but only after selected CD133 population were used [45]. More evidences are necessary to establish that these side effects are causally related to a specific subpopulation cell transfer.

So far no trial has demonstrated a significant effect of unselected BMC transfer on LV end-diastolic volumes,
suggesting a limited impact on LV remodelling after AML. Only MSC transplantation has shown improvement in regional wall motion and global LVEF and reduction in LV end-diastolic volume compared with a randomized control group that had received an intracoronary infusion of physiological saline, although a clear description of the cellular preparation is lacking in this report [44].

Autologous and allogeneic MSCs have been used in several clinical trials including Crohn disease, osteogenesis imperfecta, and graft-versus-host disease. A multicentre, phase I, placebo-controlled, double-blind trial of allogeneic MSCs has been completed and will provide information regarding the safety and efficacy of ntravenously administrated cells in patients with acute myocardial infarction (MI).

Stem cell selection and preparation prior to delivery to increase the density of the cell type of interest at the site of transplantation have been investigated not only to augment the rate of success in tissue regeneration but also to minimize the chance of cotransplanting contaminating undifferentiated stem cells and hence reduce the risk of developing a teratoma. Cell sorting using cell type-specific membrane markers, genetic methods using cell type-specific promoter or selected survival by antibiotic resistance have been proposed [53].

Table 2: Clinical studies using autologous bone marrow cells in AMI

<table>
<thead>
<tr>
<th>Study year</th>
<th>Patients (n)</th>
<th>Design</th>
<th>Follow-up (months)</th>
<th>Changes in LVEF (%)</th>
<th>Other major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strauer (2002) [39]</td>
<td>10 MNC; 10 controls</td>
<td>Non-RT IC; 5–9</td>
<td>2.8</td>
<td>2.2 ± 1.2 × 10^7</td>
<td></td>
</tr>
<tr>
<td>TOPCARE-AMI (2002) [40]</td>
<td>29 MNC; 30 CPC; 11 controls</td>
<td>No RT IC; 6–12</td>
<td>7.9 ± 4.1 × 10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez-Aviles (2004) [42]</td>
<td>20 MNC; 11 controls</td>
<td>No RT IC; 16–24</td>
<td>4.3 ± 2.3 × 10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bartunek (2005) [45]</td>
<td>19 CD133+; 16 controls</td>
<td>Non-RT IC; 7–14</td>
<td>3.9 ± 2.9 × 10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOOST (2006) [47]</td>
<td>30 NC; 30 controls</td>
<td>RCT IC; 6–12</td>
<td>3.3 ± 1.1 × 10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is equal to %, unchanged. Refer to Table 1.</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Autologous and allogeneic MSCs have been used in several clinical trials including Crohn disease, osteogenesis imperfecta, and graft-versus-host disease. A multicentre, phase I, placebo-controlled, double-blind trial of allogeneic MSCs has been completed and will provide information regarding the safety and efficacy of intravenously administered cells in patients with acute myocardial infarction (MI).
the development of noninvasive method became imperative. One such approach employed cytokine treatment, stem cell factor (SCF) and/or granulocyte colony-stimulating factor (GCSF) to mobilize endogenous BMCs and direct their integration or homing to the infarcted heart promoting repair.

Experimental studies
The first suggestion that cytokine-induced stem cell mobilization may be used to enhance cardiac repair came from studies to increase EPC levels for neovascularization in hind limb ischaemia. Mice injected with SCF and GCSF exhibited a substantial increase in the number of circulating stem cells (from 29 in nontreated controls to 7200 in cytokine-treated mice). This approach demonstrated to stimulate myogenesis and angiogenesis in the infarcted area and to improve cardiac function after AMI [62,63]. It has been postulated that GCSF may accelerate infarct healing by enhancing macrophage infiltration and matrix metalloproteinase activation, and suppress cardiomyocyte apoptosis by activating the cytoprotective STAT3 transcription factor, suggesting that stem cell–independent mechanisms may contribute to the effects of GCSF after AMI.

Human studies
Animal studies rapidly led to initiation of clinical trials to assess the ability of G-CSF to mobilize stem/progenitor cells in patients with coronary artery disease [64] and AMI [65]. G-CSF-mobilized blood from patients contained 5-fold to 100-fold higher levels of HSCs, MSCs, and EPCs, compared with nonmobilized blood; however, the ability of these cells to improve cardiac remodelling and function after AMI has been disappointing [66]. Some initial concerns regarding the safety of this approach few days after AMI was raised in clinical settings (Table 4) [67–73]. In a first clinical investigation [67], no deaths or substantial arrhythmias, aggravation of heart failure, or angina occurred during GCSF administration and a 6-month follow-up period, but infusions of GCSF mobilized peripheral blood-derived leukocytes and induced a 65% increase in serum creatine kinase-MB levels, indicative of mild myocardial damage. More seriously, 7 out of ten treated patients developed in-stent restenosis at 6 months, which prompted a premature termination of the study. GCSF has the potential to activate neutrophils, by stimulating adhesion to endothelial cells thereby influencing their recruitment at sites of inflammation and tissue injury. It was hypothesized that these systemic effects of G-CSF may have contributed to excess neointima proliferation and restenosis.

Less worrisome results came from the FIRSTLINE-AMI study [68], and the REVIVAL [69] trial where GCSF treatment after stent implantation was not associated with an enhanced rate of n-stent restenosis, or other serious adverse events: but only the FIRSTLINE-AMI
study showed some positive findings, where the beneficial effects of GCSF were magnified by an unexpected decrease in LVEF in the control group. A slightly different and complementary approach has been proposed to amplify stem cell mobilization, with positive results and no complication: after being harvested by daily G-CSF injection for 3–5 days BMC were isolated by apheresis and delivered via an over-the-wire balloon catheter.

Open issues and future developments

What is the mechanism of action?
Large debates have been promoted by the lack of any clear information on the exact mechanisms responsible for the observed benefit of this regenerative therapy. It seems that the weight of the evidence implicates mechanisms other than cellular dedifferentiation and diffusion. Different cell types, derived from tissues as varied as cord blood, adipose tissue and peripheral blood, behave similarly to bone-marrow cells after being injected directly into the heart or travelling to the ischaemic site after intravenous injection, and therefore any reported improvements in cardiac function are likely to be mediated predominantly by paracrine effects [74].

What is the best stem cell delivery route?
Multiple method of delivery for stem cells have been investigated but the optimal route is still obscure. The optimal delivery route for autologous cell transplantation not only varies according to the administered cell type but will be influenced in the future by our ability to enhance the migratory capacity of stem cells.

Systemic delivery of stem cells can be an easy method for the regeneration of the injured heart but its effectiveness is dependent on successful homing and retention of cells before the secretion of paracrine factors or transdifferentiation, or both. Alternatively, for a direct delivery to the heart, two ways have been experimented: either by an intracoronary arterial route or by injection into the ventricular wall via percutaneous endocardial, percutaneous transcoronary venous, or surgical epicardial approaches. Intracoronary delivery enables the application of a maximum dose of cells homogeneously to the site of injury although this mode is less efficient for delivery to non-perfused regions of the infarct related artery. Homing of intravenously applied progenitor cells requires their extravasation and migration to the surrounding ischaemia tissue. Although BMCs and haematopoietic stem cells can extravasate [75], this has not been shown for all cell types and larger, less motile cells, such as skeletal myoblasts may even obstruct the microcirculation, leading to embolic MI [76]. Direct injection is the preferred delivery method for chronic heart failure patients with considerable scar tissue. Cell homing signals such as SDF-1 and VEGF are expressed at low levels at late stages of
A word of caution has been raised, however. Over the past few years, cell populations expressing stem-cell marker proteins such as Kit, stem-cell antigen 1 (SCA1) and multidrug resistance protein 1 (MDR1) have been identified in the human or mouse heart, or both, albeit in minuscule quantities [80]. Although the initial evidence for an adult stem cell or progenitor-cell population in the adult heart, which could potentially be harnessed for cardiac repair, was initially welcomed with enthusiasm, scepticism has since grown. These Kit-expressing cells in tissues of solid organs, including the heart, are thought to have left the bone marrow in minuscule quantities to scavenge pathogenic molecules in peripheral tissues as part of a mechanism to promote a local innate immune response. They are not then actual heart cells but bone-marrow cells out of place. In addition, many of the cells expressing Kit that were detected in biopsied samples of adult human heart were recently reported to coexpress markers of mast cells (cells of the immune system) and to lack expression of cardiac transcription factors NKX2-5 and islet1 (Isl1), crucial markers of the cardiac progenitor cell state in fetal hearts [81]. These cells are, therefore, not cardiac progenitor cells at all.

Two approaches are currently under investigation to further investigate this issue. The first is to explant cardiac stem cells from the heart, induce their proliferation and differentiation ex vivo and engraft them back into the damaged heart [82]; and the second, which to date has received less attention, is to stimulate cardiac progenitor cells in situ to proliferate, migrate, and differentiate in the infarcted heart without ex-vivo manipulation.

Among the first approach, a clinically applicable method for the isolation and expansion of adult human cardiac stem cells from percutaneous endomyocardial biopsy specimens has been recently proposed. In culture, human cardiac stem cells self-organize into spherical clusters called cardiospheres. Human and porcine cardiospheres can differentiate into cardiac myocytes in vitro. For in-vivo experiments, infarcts in mice with severe combined immunodeficiency were created: direct injection of human cardiospheres into the infarct border zone led to myocardial regeneration by histology and to functional improvement [83].

For the second approach, a major therapeutic goal is the identification of actors that stimulate cardiac stem cells to form replacement cardiomyocytes and vascular progenitors for regeneration of the injured heart [84].

Other open issues?
The different results in different clinical trials (e.g. STEMI [47] vs. REPAIR-AMI [48]) may be the result of subtle differences in cell handling and preparation, as differences in BMC subpopulation profiles could result from different technique. Other discrepancies in these trials were evident: methods, endpoints, imaging tools evaluating cardiac function (MRI, echocardiography or angiography), patient selection, time from onset of AMI to percutaneous intervention, time from intervention to cell delivery: all these aspects may interfere with the observed responses.

The intellectual property associated with cell-based therapies is distinctly different from that associated with standard pharmaceutical development. This can contribute to the lack of major commercial investment into clinical development programmes. This has left physician researchers as the driving force for development, which encumbers them with greater responsibilities and investigative challenges. Individual researchers are traditionally competitive and tend to conduct multiple small studies rather than larger more informative ones. Moreover, the lack off unding for infrastructure and organization for multicentre trials could be a major barrier.

Conclusion
It comes immediately evident several weaknesses of what is known and what we are expecting from future investigations. Still few randomized controlled studies have been published, with different cell types and preparations, each in a small number of patients (few hundreds of patients) with different disease states, with short follow-up (from three till 18 months, but 6 months on
Cardiac regeneration

Piepoli

averaged). So although the issue on safety is generally considered ruled out, with such few numbers we still need a ward of caution, especially if we wish to be reassured about the risk of tumour formation which may take several years before it can be excluded.

Open concerns are evident also in terms of efficacy and clinical meaning. Preliminary results of human clinical trials have shown a modest improvement in the cardiac function of patients with acute myocardial ischaemia and infarct [85]. When transplantation was applied to patients with chronic myocardial disease or damage secondary to myocardial infarction the results were less definitive. However, no data are still available on crucial endpoints such as increased survival or reduced hospitalization from patients treated with cardiac regeneration. Few studies have demonstrated reduction in LV volume suggesting the lack of evident antiremodelling effect this intervention. Also the not ominously presented increases in LVEF are mostly in the range of the variability of the methodology used to assess it (imaging technique) raising some concerns regarding the real importance and clinical impact.

On the other side because the paucity of successful techniques to effectively treat heart failure, there may be mounting pressure to expedite the clinical application of cells transplant even before the mechanisms (as well as the long-term effects) are fully understood.

We should proceed in a manner that maximizes both the information gained and the safety of patients. Patients should be treated with cells only as part of randomized, controlled trials and only after they understand that neither the efficacy nor the long-term risks of this approach are established. Future trials should be powered to examine clinical end points and patients should be followed over the long term and for both beneficial and adverse effects. The enrolment of patients with a poor prognosis (i.e., large infarcts, poor left ventricular function) makes sense. They have the greatest need for therapeutic approaches and thus have the most favourable risk–benefit ratio. Demonstration of incremental benefit, as compared with conventional therapy, is easier in these populations, and subgroup analyses suggest that they are the most likely to benefit. However, we must keep in mind that the patients with too extensive myocardial damage and more advanced tissue degeneration could not be the more ideal target for this therapy because it could be less easy to get a successful homing of progenitor cells or a favourable development into functional tissues.

The enrolment of patients with heart failure who use left ventricular assist devices as a bridge to transplantation would also provide a unique opportunity to examine cellular and molecular mechanisms through analyses of cardiac tissue acquired both before cell infusion (at implantation) and after (at transplantation). Simultaneously, we must continue to support basic and translational research that can help guide clinical investigation.

In conclusion, several issues remain to be addressed in future studies. Only in large multicentre setting, with rigorous methodological criteria we will learn more about the hopes raised but not yet confirmed.

References


