Pursuing meaningful end-points for stem cell therapy assessment in ischemic cardiac disease

Maria Dorobantu, Nicoleta-Monica Popa-Fotea, Mihaela Popa, Iulia Rusu, Miruna Mihaela Micheu

Abstract

Despite optimal interventional and medical therapy, ischemic heart disease is still an important cause of morbidity and mortality worldwide. Although not included in standard of care rehabilitation, stem cell therapy (SCT) could be a solution for prompting cardiac regeneration. Multiple studies have been published from the beginning of SCT until now, but overall no unanimous conclusion could be drawn in part due to the lack of appropriate end-points. In order to appreciate the impact of SCT, multiple markers from different categories should be considered: Structural, biological, functional, physiological, but also major adverse cardiac events or quality of life. Imaging end-points are among the most used - especially left ventricle ejection fraction (LVEF) measured through different methods. Other imaging parameters are infarct size, myocardial viability and perfusion. The impact of SCT on all of the aforementioned end-points is controversial and debatable. 2D-echocardiography is widely exploited, but new approaches such as tissue Doppler, strain/strain rate or 3D-echocardiography are more accurate, especially since the latter one is comparable with the MRI gold standard estimation of LVEF. Apart from the objective parameters, there are also patient-centered evaluations to reveal the benefits of SCT, such as quality of life and performance status, the most valuable from the patient point of view. Emerging parameters investigating molecular pathways such as non-coding RNAs or inflammation cytokines have a high potential as prognostic factors. Due to the disadvantages of current techniques, new imaging methods with labelled cells tracked along their lifetime seem promising, but until now only pre-clinical trials have been conducted in humans. Overall, SCT is characterized by high heterogeneity not only in preparation, administration and type of cells, but...
also in quantification of therapy effects.

**Key words**: Stem cell therapy; Cardiac imaging techniques; Ischemic cardiac disease; Cardiac regeneration; End-points

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip**: Although multiple studies have been published on stem cell therapy (SCT) in ischemic cardiac disease, no universal conclusion regarding its clinical efficacy has been given in part due to the lack of appropriate end-points. A rightful appreciation of SCT impact should be made considering multiple parameters from different categories, either objective - evaluating structural and biological functions, or subjective - patient orientated impacting daily quality of life. Current end-points, but also novel parameters investigating molecular pathways and new imaging methods with labelled cells genetically modified are being analytically discussed in this review, disclosing high heterogeneity in SCT efficacy assessment.

---

**INTRODUCTION**

Despite modern cardiac therapies mortality and morbidity caused by ischemic heart disease (IHD) are still high. Rapid blood flow restoration improves the late outcome of patients, but it does not completely hamper myocytes loss or cardiac remodeling. Even if it is not included in the current guidelines for IHD, stem cell therapy (SCT) is one of the latest disclosures in the field.

Since the ambitious beginnings of SCT more than fifteen years ago, numerous heterogeneous results have been published regarding its outcomes. The application of SCT in patients with IHD has been proved clinically feasible and safe by clinical trials aiming heart regeneration. However promising these results may seem, SCT has yet to demonstrate clinical benefit over standard of care\(^2,\)3. The incomplete profound understanding of cardiovascular regeneration process, inconsistency in study protocols, differences from study to study clinical and biological end-points and the inappropriate routes of delivery, type and dose of cells, patients selection and randomization are some aspects which have delayed its large-scale acceptance by practitioners\(^1\).

Conducted clinical trials have been comprehensively discussed and analyzed in previous reviews\(^5\) and meta-analyses (Tables 1 and 2). Analyses were concluded on different number of randomized controlled trials (5-43) including different number of patients (262-2732 patients). Subgroup analyses were centered on various parameters such as left ventricular ejection fraction (LVEF) at enrollment, timing of stem cells (SCs) injection, number of administrated SCs and also patients’ age. Since a detailed discussion of utilized methodologies is beyond our topic of interest, we will briefly emphasize their main conclusions: SCT is safe when it is not combined with the administration of growth factors, such as granulocyte colony stimulating factor (G-CSF) that may induce stent restenosis\(^4,\)5, thrombosis\(^6\) or other adverse events\(^7\).

Leaving aside the differences in study designs, the lack of a consistent answer to the dilemma concerning the efficacy of cell therapy is sustained by the shortage of adequate end-points and by the shortcomings in evaluating these end-points. Most studies used as evaluation marker LVEF, but it is not sufficient, taking into account that more than 50% of heart failures (HF) caused by IHD have a normal ejection fraction (EF). Other surrogate parameters investigated were: Left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV), infarct size, myocardial perfusion and viability.

So, how can we properly assess the effects of SCT? The first to start with are hard clinical end-points (such as all-cause mortality or cause-specific mortality) employed to conclude whether functional improvement indeed translates into increased survival and reduced morbidity. At that point, other end-points including reinfarction, needed for revascularization and HF worsening can be taken into account. Because the hard primary end-points imply a large number of patients and a long surveillance, composite end-points are an option that overcomes the drawbacks of single end-points. Composite end-points increase the sensitivity of the study, but must be defined in the following manner: Each parameter has to be associated with the primary objective and quantified hierarchically based on its global importance. The foremost disadvantage of composite end-points is heterogeneity in the clinical relevance of the included markers. A solution to counterbalance this inconvenient is to assign a value to each end-point according to its importance, e.g., reinfarction-1, hospitalization for heart failure 0.1, etc. This type of hierarchical evaluation proposed by Finkelstein and Schoenfeld\(^8\) - although controversial - reduces the needed number of included subjects to prove clinical efficiency. For example, a trial can have the following composite end-point with the outcomes ordered by relative severity: Cardiovascular mortality, hospitalization for HF decompensation, 6-min walk test and LVEF or LVESV. Another strategy that may be used for a better assessment is evaluation through multiple parameters from different categories\(^9\). Structural measurements (the most frequently utilized group), include LVEF, LVEDV, LVESV, stroke volume, infarct size area, myocardial viability or myocardial perfusion; Biological markers: Brain natriuretic peptide, troponins, cytokines, short and long non-coding RNAs; Physiological determinants:Loading pressures, pressure-volume curves, diastolic function; Functional capacity or...
| Ref.                  | Included studies | Cell type     | Pathology | Mean change in LVEF | Other outcomes                                      |
|----------------------|------------------|---------------|-----------|---------------------|-----------------------------------------------------|
| Hristov et al. (2007)| 5 RCTs 482 subjects | BMMNCs       | AMI       | 4.21% ($P < 0.00001$) | Reduced infarct size                                  |
| Abdel Latif et al. (2007) | 18 trials (RCTs/CSs 999 subjects | BMMNCs, MSCs | AMI       | 3.66% ($P < 0.01$)   | Reduced LVESV                                         |
| Lipinski et al. (2007) | 10 trials (RCTs/CSs 698 subjects | BMMNCs, progenitor cells | AMI       | 3% ($P < 0.01$)      | Reduced infarct size, Reduced LVESV                 |
| Martin Rendon et al. (2008) | 13 RCTs | BMMNCs       | AMI       | 2.99%               | Reduced LVESV                                         |
| Zhang et al. (2009)   | 7 RCTs 660 subjects | BMMNCs       | AMI       | 4.63% ($P = 0.01$)   | Reduced infarct size                                  |
| Bai et al. (2010)     | 10 RCTs 814 subjects | BMMNCs       | AMI       | 3.79%               | Reduced infarct size                                  |
| Takagi et al. (2011)  | 15 RCTs 877 subjects | BMMNCs       | AMI       | 2.87% ($P < 0.00001$) | Reduced LVESV, Reduced LVESV                         |
| Kuswardhani et al. (2011) | 10 RCTs 906 subjects | BMMNCs, BMCs, MSCs | AMI       | 2.07% ($P = 0.008$)  | Reduced infarct size, Reduced LVESV                 |
| Clifford et al. (2012) | 33 RCTs 1765 subjects | BMMNCs, BM-CD34+ | AMI       | 2.87% maintained at 12-61 mo | Reduced LVESV, Reduced LVESV, Reduced infarct size |
| Zimmet et al. (2012)  | 29 RCTs 1830 subjects | BMMNCs       | AMI       | 2.70% ($P < 0.01$)   | No reduced LVESV                                     |
| Chen et al. (2013)    | 5 RCTs 510 subjects | BMMNCs       | AMI       | 4.18% ($P = 0.002$)  | No reduced LVESV                                     |
| Jeong et al. (2013)   | 17 RCTs 1072 patients | BMMNCs       | AMI       | 2.51% ($P = 0.002$)  | No reduced LVESV                                     |
| Delewi et al. (2013)  | 24 RCTs 1624 subjects | BMMNCs, BM-CD34+ | AMI       | 2.23% ($P < 0.01$)   | Reduced LVESV at 6 and 12 mo, Reduced recurrent AMI |
| Jong et al. (2014)    | 30 RCTs 2037 subjects | BMMNCs, MSCs, BM progenitor cells | AMI       | 2.10% ($P = 0.004$)  | Reduced LVESV                                           |
| Liu et al. (2014)     | 8 RCTs 262 subjects | MSCs         | AMI       | 3.17% ($P = 0.02$)   | A trend toward reduced LVESV, Reduced MACeS        |
| Delewi et al. (2014)  | 16 RCTs 1641 subjects | BMMNCs, CD34+/CXCR4+ | AMI       | 2.55% ($P < 0.001$)  | Reduced LVESV                                          |
| Gyöngyösi et al. (2015) | 12 RCTs 1252 subjects | BMMNCs, nucleated BMMCs, BM-CD34+ | AMI       | No improvement | No impact on MACE, No reduction on LVESV/LVEDV (MRI) |
| Fisher et al. (2015)  | 41 RCTs 2732 subjects | BMMNCs       | AMI       | No improvement in LVEF measured by MRI; 2%-5% increase by echo, PET CT and LV angiography | No effect on morbidity, quality of life/performance |
| Cong et al. (2015)    | 17 RCTs 1393 subjects | BMMNCs       | AMI       | 2.74% ($P < 0.00001, 3-6 mo) 5.1% ($P < 0.00001, 12 mo) | Reduced LVESV at 3-6 mo, Reduced WMST at 3-6 mo |
Dorobantu M et al. Cell therapy end-points for ischemic disease

Lee et al\textsuperscript{[118]} (2016) 43 RCTs 2635 subjects BMMNCs AMI 2.75% (P < 0.001) 6 mo No reduced infarct size at 6 mo Reduced infarct size at 1 yr Reduced infarct size at 3 or 5 yr No reduced mortality at 6 mo and 1 yr Reduced all-cause mortality at 5 yr

BM-CD133+ BM-CD34+ MSCs

2.75% (P < 0.001) 6 mo
1.34% (P = 0.03) 1 yr
No reduction at 3 and 5 yr

AMI: Acute myocardial infarction; BM: Bone marrow; BMCs: Bone marrow cells; BMMNCs: Bone marrow mononuclear cells; CSs: Cohort studies; CXCR4: Chemokine receptor type 4; BM-EPC: Bone marrow endothelial progenitor cells; LVEDV: Left ventricular end-diastolic volume; LVEF: Left ventricular ejection fraction; LVESV: Left ventricular end-systolic volume; MACE: Major adverse cardiac events; MSCs: Mesenchymal stem cells; PMCs: Peripheral mononuclear cells; RCTs: Randomized controlled trials; WMSI: Wall motion score index.

Table 2 Meta-analysis evaluating left ventricular ejection fraction and other outcomes in chronic, or chronic and acute settings

| Ref. | Included studies | Cell type | Pathology | Mean change in LVEF | Other outcomes |
|------|------------------|-----------|-----------|---------------------|---------------|
| Wen et al\textsuperscript{[119]} (2011) | 8 RCTs 307 subjects | BMMNCs BM-CD34+ | CIHD | 8.40% (P < 0.01) | Reduced LVESV Reduced LVEDV |
| Zhao et al\textsuperscript{[120]} (2011) | 10 RCTs 422 subjects | BM-CD34+ / CD133+ BMMNCs CPCs | CIHD | 4.02% | Reduced LVEDV Reduced LVEDV |
| Donndorf et al\textsuperscript{[121]} (2011) | 6 trials (4 RCTs and 2 CSs) 179 subjects | BMMNCs BM-CD34+ BM-CD133+ | CIHD | 5.40% (P = 0.09) | No reduced LVESV No reduced MACEs |
| Jeevanantham et al\textsuperscript{[122]} (2012) | 50 trials (RCTs, CSs) 2625 subjects | BMMNCs BM-CD133+ and/or BM-CD34+ MSCs | AMI CIHD | 3.96% (P < 0.0001) | Reduced infarct size Reduced LVESV |
| Jiang et al\textsuperscript{[123]} (2010) | 18 RCTs 980 subjects | BMCs BMMNCs MSCs and EPCs | AMI or CIHD | 2.93% (P = 0.00001) | Reduced LVESV Reduced LVEDV Reduced infarct area |
| Cheng et al\textsuperscript{[124]} (2013) | 5 RCTs 210 subjects | BMMNCs SM | Chronic ischemic HF | No significant increase Increased 6-min walk distance | Improved MLHF score Reduced NYHA class |
| Kandala et al\textsuperscript{[125]} (2013) | 10 RCTs | Unselected BMCs | CIHD | 4.48% | No reduce in all-cause mortality Reduced LVESV |
| Sadat et al\textsuperscript{[126]} (2014) | 32 trials (24 RCTs and 8 non-RCTs) 2306 subjects | Enriched BMCs BMMNCs | ACS and CAD/HF | 4.6 ± 0.7 (P < 0.05) | Reduced LVEDV Improved perfusion |
| Xu et al\textsuperscript{[127]} (2014) | 19 RCTs 886 subjects | BM-CD34+ BM-CD133+ CPCs HSCs MSCs Circulating CPCs Peripheral blood | CIHD | 3.54% (P < 0.001) | Reduced LVESV No reduced LVEDV |
| Tian et al\textsuperscript{[128]} (2014) | 11 RCTs 492 subjects | BM-CD34+ BM-CD133+ ALDH | CIHD | 4.91% (P < 0.00001) | Reduced LVEDV Reduced LVESV |
| Fisher et al\textsuperscript{[129]} (2014) | 23 RCTs 1255 subjects | BM-CD34+ BM-CD133+ CPCs HSCs MSCs | CIHD | 2.62% (P = 0.02, ≥ 12 mo) | Reduced mortality Reduced hospitalization HF (≥ 12 mo) No effect on mortality, rehospitalization for HF at short term (< 12 mo) |
performance status: 6-min walk test, maximal oxygen consumption (VO2 max) - the evaluation category with the most important impact from the patient point of view.

Quality of life
Major Adverse Cardiac Event (MACE) composite endpoint with no strictly delimited parameters.

STRUCTURAL END-POINTS
While being the most currently used, imaging techniques are extremely useful for assessing mainly the structural effects of SCT. The majority of studies concentrated on the following outcomes: LVEF, infarct size, myocardial perfusion and viability.

LVEF and left ventricular volumes
The generally measured end-point for assessing SCT outcomes is LVEF. The first clinical studies utilized unselected mononuclear bone marrow or peripheral SCs injected intracoronary. Meta-analyses dealing with these type of cells in acute myocardial infarction (AMI)
settings showed a modest increase in LVEF evaluated by various methods, between 2% to 5% [10,11,12]. One of the arguments against SCT was that the observed differences, albeit being statistically significant, had no clinical benefits. Although the LVEF recovery was small in the early period and not every time sustained, it may induce long-term positive outcomes. In this regard, it is mandatory to assess the effect of SCT on long-term, but few trials extended the follow-up after the period of one year [13-15]. An additional key aspect depicted by REPAIR-AMI was the importance of timing from AMI until cell injection. It seems that later infusion of SCs has a better outcome (i.e., LVEF) compared with the treatment administered within 4 d. This may be explained by the hostile environment which hampers cell viability due to the presence of inflammatory cells recruited in the injured area; on the other hand, a prolonged interval after AMI is inappropriate for cell transplant as a scar tissue forms and the lack of a proper vascularization also impairs SCs survival. Different imaging techniques were used to determine LVEF: Left ventricular (LV) angiography, radionuclide ventriculography, echocardiography, gated Single-Photon Emission Computed Tomography (gated-SPECT) or magnetic resonance imaging (MRI). The most accurate method to quantify LV volumes and EF is MRI and more recently, 3D-echography [16]. In Fisher’s meta-analysis it can be seen that LVEF improved in the studies that employed echography, gated SPECT or ventriculography, but not in the trials that used MRI imaging [17]. LVEF increase is a time-dependent process; some meta-analyses investigating SCT in AMI exposed an enhancement in LVEF on short-term, but not on the long-term, explained at least in part by the increase in LV volumes over time [18].

One aspect being imputed to cell based therapy and an important drawback is the targeted population, the included subjects being not very sick, with baseline LVEF around 50%. The largest trial in AMI settings (BAMI, NCT01569178) is planning to shed light and answer the question if autologous unfractionated bone-marrow offers supplemental advantages on top of AMI standard of care [19]. Unfortunately, there are no such studies on HF or chronic myocardial ischemia.

### Myocardial deformation

The standardization of other modern techniques such as strain/strain rate or tissue Doppler echocardiography are mandatory requests to find a more sensitive and specific marker for SCT outcomes. There are already small clinical trials indicating that tissue [20] and strain Doppler [21] assessment of regional systolic function might be more sensitive than global LVEF for the evaluation of SCT after AMI. The concept of myocardial strain was extended from echocardiography also to MRI detecting subtle improvements in myocardial function earlier than commonly used methods for myocardial function assessment. Myocardial MRI strain imaging has been evaluated only in one study, but when assessed showed significant increment in circumferential strain in the myocardial segments adjacent to the infarction area [22].

### Infarct size

There are several techniques that allow the quantification of the infarction area, either directly, such as nuclear imaging with Positron Emission Tomography-PET or SPECT, contrast-enhanced MRI, or indirectly, appreciating the extent of LV impairment (cine MRI, 2D-3D echocardiography, LV angiography). From the aforementioned approaches the most accurate is contrast-enhanced MRI and the only one capable to distinguish the transmural from the subendocardial infarction. Although the majority of studies using MRI assessment proved no decrease of infarct size compared with placebo [23,24], there was one trial that interestingly showed a greater reduction in the infarction area in patients having a higher percentage of CD45+CD31+ cells in the bone marrow. These findings endorse the conclusion that cells’ phenotype, as well as their functional capacity are key determinants of individual responses to SCT [25].

Studies using SPECT disclosed a significantly reduced number of myocardial scar segments per patient in case of intracoronary infusion of an autologous population of culture expanded mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) [26], while transplantation of unselected bone marrow mononuclear cells had no impact on the above cited parameter [27].

### Myocardial viability

The current imaging techniques for appraising myocardial viability are: Nuclear imaging (PET or SPECT), low-dose dobutamine echocardiography and MRI. From the practical point of view, an ideal device is the one that provides real-time information as regards myocardial viability and allows targeted cell delivery. Cardiac electromechanical mapping solved this problem and significantly correlates with PET, because in the same time it can be seen if electrical activation translates into mechanical contraction; studies relying on this method showed that SCT increases the local shortening in the infarcted area [28,29]. Nuclear perfusion imaging, mainly PET-CT has been considered a gold standard for the detection of viable myocardium. Other techniques such as SPECT reported no change compared with control groups [30-32]. On the other hand, studies using 18F-FDG PET indicated a gain in myocardial viability [29,33-36] which was not every time confirmed in trials with low-dose dobutamine echocardiography, possibly due to the fact that severe damaged myocardium can still preserve glucose uptake whilst the contractility is lost [27]. Low-dose dobutamine echocardiography showed no improvement in the contractile reserve of patients with AMI and mononuclear bone marrow SCT compared to the control group [37].
with the non-treated\cite{34,36}, however when MSCs and EPCs were used an increased number of viable segments was observed\cite{26}.

**Myocardial perfusion**

The available tools for myocardial perfusion evaluation are: MRI (rest first-pass perfusion and late gadolinium enhancement imaging), nuclear imaging (SPECT, PET) and contrast echocardiography. The majority of studies that used SPECT showed a slight increase in myocardial perfusion\cite{39,40}, although more specific and sensitive methods such as PET\cite{41,42} failed to prove significant difference between SCT and control groups. MRI studies also revealed no improve in perfusion after SCT\cite{33,43,44}. PET has the additional benefit of quantifying myocardial blood flow (MBF) using $^{13}$N-ammonia, $^{15}$O water or $^{82}$Rb. MBF quantification is a useful tool to identify patients with balanced triple vessel disease and to diagnose endothelial dysfunction\cite{45}. One study successfully assessed cardiac perfusion, metabolism, and function in patients treated with intracoronary injection of endothelial progenitors using $^{13}$N-ammonia and $^{18}$F-FDG PET\cite{42}, showing that selected bone marrow-derived CD133+ cells significantly reduced the number of scarred segments and infarct size along with an increase in MBF. Larger studies are required in order to certify the diagnostic and prognostic value of quantitative MBF in relation to SCT.

**BIOLOGICAL END-POINTS**

N-terminal pro-brain type natriuretic peptide (NT-proBNP) is an important biomarker in IHD. However, when it comes to compare patients treated with SCT to those without treatment, it does not significantly differ. On a short-term follow-up intracoronary bone marrow mononuclear cells (BMMNCs) therapy does not have any impact on NT-proBNP or inflammatory markers such as IL-6, high-sensitivity CRP (C reactive protein) and TNF-α in ST elevation myocardial infarction (STEMI) patients\cite{46}. Furthermore, on long-term, it was shown that patients with chronic ischemic HF treated with intramyocardial BMMNCs therapy during coronary artery bypass grafting had not had different levels of NT-proBNP from control patients\cite{47}. In addition, in a 5-year follow-up study, NT-proBNP used as an objective marker for cardiac function remained significantly low in patients treated with circulating or bone marrow-derived progenitor cells\cite{48}. Even though it was believed that troponin elevation during SCs harvesting and intramyocardial delivery has no meaningful impact on clinical outcome\cite{48}, latest published data support the hypothesis that high-sensitive troponin T serum levels inversely correlate with cell retention and may regulate the response to SCT in patients with post-infarction HF\cite{49}.

Recent data revealed the implication of several cytokines in the progenitor cell evolution and cardiac function in experimental models, but there is only one study which analyzed it in humans. Shahrivari et al.\cite{50} demonstrated that increased platelet-derived growth factor BB (PDGF-BB) glycoprotein in the peripheral blood is related to increased bone-marrow function, while high levels of IL-6 is related with bone-marrow impairment. In the same vision, supporting the hypothesis that SCT has a role in maintaining the balance of inflammatory markers, Alastalo et al.\cite{51} investigated the implication of cytokines in STEMI patients undergoing SCT by evaluating the levels of IL-4, IL-10, IL-13, IL-1ß, IL-6, TNF-α and IFN-γ; obtained results pointed to the conclusion that SCT reduces inflammatory cytokines and promotes anti-inflammatory markers.

**PHYSIOLOGICAL END-POINTS**

Diastolic dysfunction was associated with neurohormonal activation and also with the severity of coronary disease evaluated by angiography, being in consequence an independent predictor of post-AMI prognosis\cite{52}. Although the majority of studies with SCT in AMI focused their attention on LVEF and LV remodeling evaluation, few of them investigated diastolic function with heterogeneous findings. In the BOOST study, E/A ratio, deceleration time, diastolic tissue velocities and isovolumic relaxation time were determined; among these, the only parameter positively influenced was E/A ratio, which is not satisfactory taking into account that LV filling patterns have a U-shaped relation with LV diastolic function\cite{53}. This favorable result was maintained only in the first 18 mo\cite{54}, but not at 5 years\cite{55}.

E/e’ has a more linear relation to LV filling pressure and therefore is recommended for the evaluation of LV diastolic function. Four months after cell transplant, Herbots et al.\cite{56} did not identify a statistically significant difference between groups with reference to E/e’, but another trial proved an improvement, despite the fact that no comparison with the placebo group was completed\cite{59}. On the other hand, Beitnes et al.\cite{60} reported a constant decrease in E/A and E/e’ ratio along with an increase in deceleration time in both groups, independently of SCT. A meta-analysis that included 6 trials with a total of 365 patients revealed a superior improvement in E/e’ ratio at 1 year in the treated group compared with control\cite{56}. In the study conducted by Yao including patients with chronic myocardial disease it was disclosed that even though there were no significant differences between groups in LV volumes, infarct size or myocardial perfusion, there was an overall effect of SCT on E/A, E’/A ratio and isovolumic relaxation time at 6 mo follow-up\cite{57}.

There are also a series of negative studies, as ASTAMI, where reduced E/A ratio, increased deceleration time and reduced E/e’ were observed in both groups, probably reflecting a decrease in filling pressure\cite{58}.

**FUNCTIONAL CAPACITY**

Apart from the classic parameters, a small number of trials also included patient-centered end-points evaluating the impact of SCT on status performance and quality of
life.

An indicator of functional improvement after myocardial infarction is the performance status which has been assessed in certain studies by means of the New York Heart Association (NYHA) Functional Classification. The published results did not show improvements in NYHA class between the group receiving cell therapy and the control group[59-63], but the heterogeneity index of the studies was high ($I^2 = 80\%$) making interpretation questionable[17].

Other manners to address performance are exercise tests: Treadmill test[40], 6-min walk test[64], bicycle ergometer[59] and symptom-limited maximal exercise test[65]. A meta-analysis including the previous types of tests exposed no improved exercise tolerance. From the analyzed trials only one displayed higher $O_2$ consumption and better ventilatory response to exercise[66], Meta-analysis conducted by Fisher et al[67] explored, among other parameters, the effect of SCT on exercise capacity; the authors concluded that patients undergoing SCT had greater performance status, but the measurement scales were different impeding correct interpretation.

However, the relationship between SCT and exercise is bidirectional: It is not only that the transplant patient can produce changes in performance status, also exercise influences cells’ behavior and clinical outcome. Preclinical studies demonstrated that exercise could increase exogenously infused bone marrow cell retention in mouse myocardium, suggesting that exercise may support SCT[61]. Hence, we should display more interest in addressing this issue.

QUALITY OF LIFE

Whereas 5 trials have examined the quality of life (QOL) after SCs transplantation in AMI on short term, there is still lack of information on the long-term, just one study reporting end-points at 12 mo[68]. In this small study with only 26 participants, QOL was significantly improved at one year follow-up. From the 5 trials mentioned above, 3 evaluated QOL with Minnesota Living with Heart Failure Questionnaire (MLHFQ)[64,66,69] and 2 trials with the Short Form 36 Health Survey[59,62]. A meta-analysis including only 3 of the 5 trials - due to missing data - did not show a significant improvement on short term in the life quality of treated patients compared with the control[70]. There were also a few studies in chronic IHD or HF, but due to the fact that the results have not been presented quantitatively but only descriptively, no conclusions can be drawn[71,72].

Angina frequency is one of the disease-specific health-related QOL (HRQOL) items measured using dedicated instruments[72], therefore there is no wonder that it has been widely assessed in relation to SCT.

Concerning the frequency of angina, all published trials in unanimity showed a reduction in the number of episodes, reported either by a reduction in the frequency of angina episodes per week[40] or as the frequency of angina at short-term follow-up[71,79]. A valuable parameter in evaluating the clinical assessments of SCT would be the psychological dimension, proved to be an essential factor in cardiac rehabilitation[76]. A pilot study evaluated the impact of psychological and behavioral factors in patients with AMI undergoing SCT indicated that psychological factors should be taken into consideration in evaluation of the response to SCT[77].

MACE

One commonly evaluated composite end-point in cardiology research is MACE. Although created to evaluate effectiveness and safety, it is study variable as the outcomes differ from trial to trial and there is no universal definition. Meta-analyses proved that MACE creates high heterogeneity in conclusions between studies according to the parameters taken into account[78].

There is some evidence indicating that even small improvement in LVEF in AMI patients treated with SCT reduces cardiovascular mortality in the long term. REPAIR-AMI trial at 2 and 5 years follow-up showed beneficial clinical effects in cardiovascular mortality and rehospitalization for HF (4 deaths/100 patients in treated group compared with 14 deaths/100 in the placebo group)[79]. One important limitation of the mentioned study is related to the small number of events (15 deaths in the placebo group and 7 in BMMNCs group during the 5-year follow-up interval). Of note, enrolled patients had a mean baseline LVEF above 45%, meaning that patients with severe impaired systolic function have not been included, namely the cohort at the highest risk for future adverse cardiovascular events.

Unlike the majority of trials where SCT was applied in AMI settings, most recent meta-analyses conducted in chronic IHD and HF pointed out beneficial clinical effects in long term mortality, without losing sight that the quality of evidence is low[80,81]. In refractory angina patients candidates for revascularization SCT improved the scores for angina, myocardial perfusion and a composite end-point MACE (myocardial infarction, cardiac-related hospitalization and mortality)[82].

EMERGING PARAMETRES

In recent studies, it was shown a great interest toward microRNAs (miRNAs) as clinical biomarkers in cardiovascular disease[83]. MiRNAs are small non-coding RNA molecules implicated in gene expression regulation by suppressing the translation of their target messenger RNAs (mRNAs); they can be released in circulation, easily detected in the plasma and quantified by real-time PCR or microarrays, therefore not hard to obtain and analyzed[84]. Lately, miRNAs have proved their implication in cardiogenesis and regeneration of cardiac tissue, so it is likely to have a possible impact in patients undergoing SCT.

Schulte et al[85] outlined the perspective use of miRNAs as biomarkers for diagnosis and prognosis of
HF patients. In a recent published study, Karakas et al[86] evaluated the prognostic value of circulating miRNAs in a cohort of 1112 patients with acute coronary syndrome or stable angina pectoris and pointed out the potential of miRNAs to predict cardiovascular death in these patients. There has been only one study which performed profiling and validation of circulating miRNAs related to MACE in patients with STEMI, demonstrating that specific miRNAs reflect the clinical outcome after STEMI[87].

Long non-coding RNAs (IncRNAs) were less studied than miRNAs in cardiac pathology[88]. Still, it was demonstrated that IncRNAs can predict the prognosis in patients with AMI and HF[89,90]. One of the advantages of IncRNAs is their ability to differentiate between ischemic and non-ischemic HF compared with miRNA. Also, IncRNAs expression differs with hemodynamic conditions, suggesting that it could be a potential biomarker in evaluating myocardial recovery under mechanical circulatory support[89,90].

Another parameter to consider could be the impact of SCT on endothelial function. There is robust evidence showing that MSCs restore endothelial progenitor cell function and vasculogenesis, thus improving flow-mediated dilatation, decreasing vascular endothelial growth-factor (VEGF) while concomitantly increasing EPC-CFU<sub>sm</sub> (endothelial progenitor cell colony-forming units smooth muscle)<sup>[91]</sup>.

**IMAGING MODALITIES TO BE TRANSLATED FROM BENCH TO BEDSIDE**

Different from the presented imaging techniques that assess only marginally and indirectly the fate of transplanted cells, the ideal imaging modality should be able to provide information about their engraftment, survival, proliferation, differentiation, maturation and integration. Labelling strategies for adequate in vivo surveillance and cell tracking is the key to solve some unanswered questions about SCT in cardiovascular diseases and it includes superparamagnetic-iron oxide (SPIO) MRI, direct labelling and reporter genes.

Direct imaging implies cells incubation with various probes that enter the cell by endocytosis (SPIOs), transporter uptake (¹⁸F-FDG) or passive diffusion (¹¹¹In-ox). Direct labelling of cells using magnetic resonance agents tracks cells and gives details about their biology. SPIO persists in the cells and along with the high resolution and good tissue contrasts make MRI a suitable tool for cell tracking<sup>[92]</sup>. A drawback of MRI-SPIO worth considering in long-term imaging is the uptake of the contrast agent in the resident macrophages that can show a false-positive increase of the signal as if there would be high engraftment and survival. This inconvenient of SPIOs accumulation in macrophages is of interest in studies investigating inflammation sites<sup>[93]</sup>. What is more, even if there is little or no impact of SPIO as regards cells viability and proliferation capacity, some evidence indicate that SPIO labeling of MSCs impedes cellular differentiation down a specific pathway (i.e., chondrogenesis but not adipogenesis or osteogenesis)<sup>[94]</sup>. Nevertheless, this effect must be product dependent because there are other iron-based products approved that do not illicit harmful effects neither on the hematopoietic, nor on the BM MSCs<sup>[95]</sup>.

Direct radionuclide labelling is widely spread, has high sensitivity, but poor spatial resolution. SPECT and PET are the most frequently employed to describe bio distribution. When cells are injected into the coronary artery or vein by using the stop-flow technique, the retention of BMMNCs is 10.3% and 3.1%, respectively<sup>[96]</sup>. When CD34<sup>+</sup> cells are labelled with <sup>99m</sup>Tc-HMPAO retention rises at 19% at 18 h post-injection<sup>[97]</sup>. But an important disadvantage is the short half-lives of the used radiotracers that does not allow long-term follow-up.<sup>[98]</sup> In-oxine having a T<sub>1/2</sub> = 2.8 days lengthens the total tracking duration to 3-4 d, pointing a level of 2% cell retain<sup>[99]</sup>.

Reporter gene imaging needs transfection or transduction with reporter gene constructs. After transcription and translation of the reporter gene under the control of a promoter, reporter proteins cumulate into the cell. Upon insertion of a probe specifically to the reporter gene (optical, radio-labelled), the signal starts to be generated and the cells are detected with different imaging modalities (PET, MRI, SPECT, CT, bioluminescence or fluorescence imaging). Reporter genes for cardiovascular SCT seem to be an ideal approach, but apart from one study<sup>[100]</sup> that applied it to cytolytic CD8<sup>+</sup>T<sub>cyt</sub> in a patient with glioblastoma, all other trials were preclinical<sup>[100,101]</sup>. Not only distribution and proliferation can be assessed with reporter genes, but also differentiation and maturation of cells using a promoter for a differentiation-specific locus, such as sodium-iodide symporter<sup>[102]</sup>. On the other hand, reporter gene technique implies genetic modification that seriously increases the risk for mutagenesis. In order to impede inappropriate insertion and prompt targeted insertion, novel gene editing methods can be used such as transcription activator-like effector nuclease (TALEN) or clustered regularly interspaced short palindromic repeats (CRISPR).

All the aforesaid imaging modalities are valuable tools for in vivo surveillance and cell tracking waiting to be refined and translated in clinical practice.

**CURRENT RECOMMENDATIONS REGARDING SCT**

Recommendations in the field of SCT target preclinical and clinical research and are of great value in the perpetual quest to overcome the above mentioned hurdles. In accordance with the requirements for good clinical practice and clinical research established by the regulatory bodies in the United States and Europe, phase I clinical trials should not only consider a variety of efficacy domains, but also should assess the potential benefits of SCT while not focusing on the statistical significance of P value<sup>[103]</sup>. Furthermore, they should
include many primary surrogate end-points such as functional and structural measures, biomarkers, quality of life and functional capacity (Figure 1). More precisely, phase II clinical trials have the purpose of generating hypotheses to be used in the appropriate design of pivotal confirmatory phase III clinical trials\cite{1,2}. Finally, the utilization of hard clinically meaningful end-points is compulsory for the assessment of whether functional improvement positively translates into heightened survival and reduced morbidity\cite{103}. In this regard, phase III trials should test hard clinical end-points such as all-cause mortality or cause specific mortality, improved survival, reduced clinical events/number of hospitalizations which have applicability in the daily clinical practice. Also, well-designed phase III trials should evaluate subjective clinically relevant end-points as symptom score and HRQOL\cite{1,2}.

Another recommendation is related to the techniques that should be used for surrogate end-points measurements; accordingly, the most reproducible techniques are endorsed (e.g., MRI, PET), while centralized analysis should be settled by core laboratories\cite{1}. Nonetheless, patient selection is of the essence. When designing a new clinical trial, confounders such as gender, age, comorbidities, concomitant medications, disease vulnerability and severity should always be taken into consideration, if possible by means of predictive scores of outcomes\cite{1,103}. The focus for inclusion/exclusion criteria in the trial should be on subpopulations with poor prognosis, as they are the target patient that could benefit the most from SCT\cite{1}.

New “mechanistic” end-points are required in order to better understand the regeneration capacity of the adult mammalian heart and to validate hypothesis on SCs mechanisms of action; these novel end-points should be integrated in traditional safety and efficacy end-points - either surrogate or clinical, only after proper validation in the preclinical research field and in agreement with regulatory recommendations\cite{1}.

With regard to the aforementioned recommendations, in their position paper issued on May 2017, TACTICS highlights the challenges in the field of cardiovascular regenerative medicine for the next decade. Among their global aims are achieving uniformity and, consequently, meeting the required norms for clinical research of animal models for cardiovascular research; using collective achievement of phase III multicenter clinical trials that are optimally designed to improve standard of care in cardiovascular medicine and demonstrate the clinical efficiency of SCT. The last but not the least goal is to certify implementation of accepted SCT via transnational standardization of regulatory requirements.

Regarding initiation of future autologous bone marrow cells clinical trials in AMI, the recommendation is to await results from on-going BAMI trial (NCT01569178)- multicenter, randomized, controlled, phase III study-designed to assess efficacy of SCT with concern to morbidity and mortality in patients with reduced LVEF after successful reperfusion when compared to a control group of patients undergoing best medical care.

As for clinical trials in HF, cardiopoietic cells-either primary or engineered - should be used. Taking into consideration the documented safety of SCT approaches, trials evaluating repeated administration should be...
studied in order to enhance long term clinical outcome [19].

CONCLUSION
SCT in ischemic cardiac disease is characterized by high heterogeneity in the assessment of therapeutic benefits due in part to the imprecise end-points. Apart from the classic structural parameters, new emerging imaging or biological markers promise to enlighten the field of cardiac regeneration offering less debatable results.

REFERENCES
1. Fernández-Avildes F, Sanz-Ruíz R, Climent AM, Badimon L, Bolli R, Charron D, Fuster V, Janssens S, Kastrup J, Kim HS, Lüscher TF, Martin JF, Menasché P, Simari RD, Stone GW, Tziric A, Willerson JT, Wu JC. TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovacular Syndromes) Writing Group; Authors/Task Force Members. Chairpersons; Basic Research Subcommittee; Translational Research Subcommittee; Challenges of Cardiovacular Regenerative Medicine Subcommittee; Tissue Engineering Subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee; Clinical Trials Subcommittee; Regulatory and funding strategies subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee. Global position paper on cardiovascular regenerative medicine. Eur Heart J 2017; 38: 2532-2546 [PMID: 28575280 DOI: 10.1093/eurheartj/ ehx248].

2. Banovic M, Loncar Z, Behfar A, Vanderheyden M, Beleslin S, Zeiher A, Metra M, Tziric A, Bartunek J. Endpoints in stem cell trials in ischemic heart failure. Stem Cell Res Ther 2015; 6: 159 [PMID: 26319401 DOI: 10.1186/s13287-015-0143-9].

3. Micheu MM, Dorobantu M. Fifteen years of bone marrow mononuclear cell therapy in acute myocardial infarction. World J Stem Cells 2017; 9: 68-76 [PMID: 28491241 DOI: 10.4252/wjst.v9.i4.68].

4. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, Kim YJ, Soo Lee D, Sohn DW, Oh BH, Lee MM, Park YB. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function in ischemic heart failure. Int J Cardiol 2007; 127: 248-249 [PMID: 17275936 DOI: 10.1016/j.ijcard.2006.11.004].

5. Kleinwender C, Hofmann R, Krypta A, Gabriel C, Leisch F. Late stent thrombosis after transcoronary transplantation of granulocyte-colony stimulating factor-mobilized peripheral blood stem cells in patients with chronic refractory ischemic heart disease: the G-CSF in angina patients with IHID to stimulate neovascularization (GAIN I) trial. Am Heart J 2008; 156: 954-963 [PMID: 19061712 DOI: 10.1016/j.ahj.2008.04.034].

6. Finkelstein DM, Schoenfeld DA. Combining mortality and longitudinal measures in clinical trials. Stat Med 1999; 18: 1341-1354 [PMID: 10399200 DOI: 10.1002/(ISSN)1097-0258 (19990615)18:113.CO;2-7].

7. Hare JM, Bolli R, Cooke JP, Gordon DJ, Henry TD, Perin EC, March KL, Murphy MP, Pepine CJ, Simari RD, Skaratlos SI, Traverse JH, Willerson JT, Szady AD, Taylor DA, Vojvodic RW, Yang PC, Moyé LA. Cardiovacular Cell Therapy Research Network. Phase II clinical research design in cardiology: learning the right lessons too well: observations and recommendations from the Cardiovascular Cell Therapy Research Network (CCTRN). Circulation 2013; 127: 1639-1653 [PMID: 23588961 DOI: 10.1161/CIRCULATIONAHA.112.00779].

8. Kuswardhani RA, Soejitno A. Bone marrow-derived stem cells as an adjunctive treatment for acute myocardial infarction: a systematic review and meta-analysis. Acta Med Indones 2011; 43: 168-177 [PMID: 21970926].

9. Ziemet H, Porapakkham P, Porapakkham P, Sata Y, Haas SJ, Jiescu S, Forbes A, Krum H. Short- and long-term outcomes of intracoronary and endogenously mobilized bone marrow stem cells in the treatment of ST-segment elevation myocardial infarction: a meta-analysis of randomized control trials. Eur J Heart Fail 2012; 14: 91-105 [PMID: 22065869 DOI: 10.1093/euheartj/ehr148].

10. Cong QX, Li Y, Zhao X, Dai YJ, Liu Y. Short-Term Effect of Autologous Bone Marrow Stem Cells to Treat Acute Myocardial Infarction: A Meta-Analysis of Randomized Controlled Clinical Trials. J Cardiovasc Transl Res 2015; 8: 221-231 [PMID: 25953677 DOI: 10.1007/s12265-015-9621-9].

11. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Horng B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOST model randomised controlled clinical trial. Lancet 2004; 364: 141-148 [PMID: 15246726 DOI: 10.1016/S0140-6736(04)6626-9].

12. Meyer GP, Wollert KC, Lotz J, Pirr J, Rager U, Lippolt P, Hahn A, Fichtner S, Schaefer A, Arseniev L, Ganser A, Drexler H. Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. Eur Heart J 2009; 30: 2978-2984 [PMID: 19773226 DOI: 10.1093/eurheartj/ehp374].

13. Assmus B, Rolf A, Erbs S, Elsässer A, Habersbosch W, Hambrecht R, Tillmanns H, Yu J, Corti R, Mathey DG, Hamm CW, Süsselbeck T, Torni T, Dimmeler S, Dill T, Zeiher AM, Schächinger V, REPAIR-AMI Investigators. Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circulation 2010; 3: 89-96 [PMID: 19996415 DOI: 10.1161/CIRCFAILURE.108.342343].

14. Hung J, Lang R, Flachskampf F, Sherman SK, McCulloch ML, Adams DB, Thomas J, Vannan M, Ryan T; ASE. 3D echocardiography: a review of the current status and future directions. J Am Soc Echocardiogr 2007; 20: 213-233 [PMID: 17363747 DOI: 10.1016/j.echo.2007.01.010].

15. Fisher SA, Zhang H, Doree C, Mathur A, Martin-Rendon E. Stem cell treatment for acute myocardial infarction. Cochrane Database Syst Rev 2015; (9): CD006536 [PMID: 26419913 DOI: 10.1002/14651858.CD006536.pub4].

16. de Jong R, Houtgraaf JH, Samie S, Boersma E, Duckers HJ. Intracoronary stem cell infusion after acute myocardial infarction: a meta-analysis and update on clinical trials. Circ Cardiovasc Interv 2014; 7: 156-167 [PMID: 24668227 DOI: 10.1161/CIRCINTERVENTIONS.113.001009].

17. Martur A, Fernández-Avildes F, Dimmeler S, Hauskeller C, Janssens S, Menasché P, Wojakowski W, Martin JF, Zeiher A; BAMI Investigators. The consensus of the Task Force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for the treatment of acute myocardial infarction and heart failure: update 2016. Eur Heart J 2017; 38: 2930-2935 [PMID: 28020448 DOI: 10.1093/eurheartj/ehw640].

18. Ruan W, Pan CZ, Huang GQ, Li YL, Ge JB, Shu XH. Assessment of left ventricular segmental function after autologous bone marrow stem cells transplantation in patients with acute myocardial infarction by tissue tracking and strain imaging. Chin Med J (Engl) 2005; 118: 1175-1181 [PMID: 16117861 DOI: 10.4103/0376-0860.117511].

19. Herbots L, D’hooge J, Eriglu E, Thijs D, Garanne J, Claus P, Dubois C, Theunissen K, Bogaraj J, Jens L, Kaliantsi M, Dymarkowski S, Bijnens B, Belmans A, Boogaerts M, Sutherland G, Van der Werf.
Dorobantu M et al. Cell therapy end-points for ischemic disease

F, Rademakers F, Janssens S. Improved regional function after autologous bone marrow-derived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study. *Europ Heart J* 2009; 30: 662-670 [PMID: 19106196 DOI: 10.1093/eurheartj/ehn573]

Bhatti S, Hakeem A, Taylor M, Chung E, Qusayumi AA, Ooshinski J, Pecora AL, Kereiakes D, Hor K, Mazur W. MRI strain analysis as a novel modality for the assessment of myocardial function following stem cell therapy-results from Amorecty trial. *J Cardiovasc Magn Reson* 2011; 13: P86 [DOI: 10.1186/1532-429X-13-S1-P86]

Wöhrl J, Merkle N, Mailänder V, Nusser T, Schauwecker P, von Scheidt F, Schwarz K, Bonnur M, Wiesnet M, Schrenzeimier H, Hornbach V. Results of intracoronary stem cell therapy after acute myocardial infarction. *Am J Cardiol* 2010; 105: 804-812 [PMID: 21233213 DOI: 10.1097/JAM.0b013e3181fbb986]

Leitner DM, Fischer-Rasokat U, Honold J, Seeger FH, Schächinger V, Lehmam R, Martin H, Burck I, Urbich C, Dimmeler S, Zeiher AM, Assmus B. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. *Clin Res Cardiol* 2011; 100: 925-934 [PMID: 21639231 DOI: 10.1007/s00392-011-0327-y]

Schutt RC, Trachtenberg BH, Cooke JP, Traverse JH, Henry TD, Pepine CJ, Willerson JT, Perin EC, Ellis SG, Zhao DX, Bhatnagar A, Johnstone BH, Lai D, Resende M, Ebert RF, Wu JC, Sayre SL, Orozco A, Zierold C, Simari RD, Moyé L, Cogle CR, Taylor DA; Cardiovascular Cell Therapy Research Network (CCTRN). Bone marrow characteristics associated with changes in infarct size after STEMI: a biopsychometric analysis from the CCTRN TIME trial. *Circ Res* 2015; 116: 99-107 [PMID: 25460300 DOI: 10.1161/CIRCRESAHA.113.304710]

Katritsis DG, Sotiriopoulou PA, Karvouni E, Karaboinis I, Korovessis P, Perez SA, Voridis EM, Papamichail M. Transcoronal transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Inter* 2005; 65: 321-329 [PMID: 15954106 DOI: 10.1002/ccd.20406]

Lunde K, Solheim S, Aakhus S, Aumen H, Abdelnoor M, Egeland T, Endresen K, Ibebekk A, Mangschaus A, Fjell JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjørnerem R, Brekke M, Müller C, Hoppe E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006; 355: 1199-1209 [PMID: 16990383 DOI: 10.1056/NEJMoa055706]

Perin EC, Dollmann HF, Borjevic R, Silva SA, Sousa AL, Silva GV, Mesquita CT, Belém L, Vaughn WK, Rangel FO, Assad JA, Carvalho AC, BramVV, Rossi MI, Dollmann JT. Improved exercise capacity and ischemia 6 and 12 months after transcatheter administration of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circulation* 2004; 110: I213-I218 [PMID: 15364865 DOI: 10.1161/01.acre.2004.12.018]

Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chen SL, Lunde K, Assmus B, Ersels J, Poldermans D, Bas JX. Nuclear imaging is more sensitive for the detection of viable myocardium than dobutamine echocardiography. *Nat Med Commun* 2023; 4: 375-381 [PMID: 12673165 DOI: 10.1002/nmcd.2020000000]

Fernández-Avilés F, San Román JA, García-Grande J, Fernández ME, Peñarrubia MJ, de la Fuente L, Gómez-Bueno M, Cartalapiedra A, Fernández J, Gutiérrez O, Sánchez P, Hernández C, Sanz R, García-Sancho J, Sánchez A. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. *Circ Res* 2004; 95: 742-748 [PMID: 15353665 DOI: 10.1161/01.hre.0000144798.54040.e0]

Lipice P, Krzemińska-Pakula M, Plewka M, Kuśmierek J, Ptaszyński J, Ptaszycki A, Soszna FS, Putala P, Kowalczuk A, Radomski M, Polandzak A. Impact of intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction on left ventricular perfusion and function: a 6-month follow-up gated 99mTc-MIBI single-photon emission computed tomography study. *Eur J Nucl Med Mol Imaging* 2009; 36: 587-593 [PMID: 19090777 DOI: 10.1007/s00259-008-0988-6]

Grajek S, Popiel M, Gil L, Breborowicz P, Lasik M, Czyczpowsky R, Sawiński K, Straburzyńska-Migaj E, Araszkiewicz A, Czyz A, Kozłowska-Skrzyzak M, Kornacki M. Influence of bone marrow stem cells on left ventricular perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: randomized clinical trial: Impact of bone marrow stem cell intracoronary infusion on improvement of microcirculation. *Europ Heart J* 2010; 31: 691-702 [PMID: 20022872 DOI: 10.1093/eurheartj/ehp356]

Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Denzet W, Kalantzi M, Herbots L, Sinaeuve P, Jens M, Maertens J, Rademakers F, Dymarkowski S, Heggiesen O, Van Cleempf J, Bormans G, Nyuys J, Belmans A, Mortelmans L, Boogaerts M, Van der Werf F. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet* 2006; 367: 113-121 [PMID: 16413875 DOI: 10.1016/S0140-6736(06)68780-1]

Castellani M, Colombo A, Giordano R, Pusineri E, Canzi C, Longari V, Piccaluga E, Palatresi S, Dellavedova L, Soligo D, Rebullia P, Gerundini P. The role of PET with 13N-ammonia and 18FDG in the assessment of myocardial perfusion and metabolism in patients with recent AMI and intracoronary stem cell injection. *J Nucl Med* 2010; 51: 1908-1916 [PMID: 21078804 DOI: 10.2967/jnumed.110.078469]

Robbers LF, Nijseldt R, Beek AM, Hirsch A, van der Laan AM, Delewi R, van der Vleuten PA, Tio RA, Tijssen JG, Hofmann MB, Piek
Recommendations for the evaluation of left ventricular diastolic function. J Am Coll Cardiol 2010; 55: A100.E1024 [DOI: 10.1016/S0735-1097(10)61025-X]

Al-Shehrli H, Small G CB. Cardiac CT, MR, SPECT, ECHO, and PET. What test, when? Appl Radiol 2011. Available from: URL: http://applradiology.org/articles/cardiac-cmr-spet-echo-and-pet-what-test-when

Miettenen JA, Ylitalo K, Hedberg P, Kerivinen K, Niemelä M, Säily M, Koistinen P, Savolainen ER, Ukkonen H, Pietilä M, Airaksinen KE, Knutti J, Vuotteenaho O, Mäkiäilio TH, Huikuri HV. Effects of intraocular injection of autologous bone marrow-derived stem cells on natriuretic peptides and inflammatory markers in patients with acute ST-elevation myocardial infarction. Clin Res Cardiol 2011; 100: 317-325 [PMID: 20959359 DOI: 10.1007/s00392-010-0246-3]

Lehtinen M, Pülläni T, Kankuri E, Lauerma K, Sinisalo J, Löise M, Kupari M, Vento A, Harjula A; Helsinki BMMC Collaboration. Intramyocardial bone marrow mononuclear cell transplantation in ischemic heart failure: Long-term follow-up. J Heart Lung Transplant 2015; 34: 899-905 [PMID: 25797522 DOI: 10.1016/j.healun.2015.01.989]

Povsic TJ, Losando DW, Story K, Junge CE, Schatz RA, Harrington RA, Henry TD. Incidence and clinical significance of cardiac biomarker elevation during stem cell mobilization, apheresis, and intramyocardial delivery: an analysis from ACT34-CMI. Am J Heart 2012; 164: 689-697.e3 [PMID: 23137499 DOI: 10.1016/j.ahj.2012.06.022]

Luu B, Leistner DM, Herrmann E, Seeger FH, Honold J, Fichtlscherer S, Zeiher AM, Assmus B. Minute Myocardial Injury as Measured by High-Sensitive Troponin T Serum Levels Predicts the Response to Intracoronary Infusion of Bone Marrow-Derived Mononuclear Cells in Patients With Stable Chronic Post-Infarction Heart Failure: Insights From the TOPCARD-CHD Registry. Circ Res 2017; 120: 1938-1946 [PMID: 28351842 DOI: 10.1161/CIRCRESAHA.116.309938]

Shahrivari M, Wise E, Resende M, Shahrivari M, Alestalo K, Husic M, Vento A, Harjula A; Helsinki BMMC Collaboration. Intramyocardial bone marrow mononuclear cell transplantation in ischemic heart failure: Long-term follow-up. J Heart Lung Transplant 2015; 34: 899-905 [PMID: 25797522 DOI: 10.1016/j.healun.2015.01.989]

Hirsch A, Niijeltd R, van de Vleuten PA, Tijssen JG, van der Giessen WJ, Tio RA, Waltenberger J, ten Berg JM, Doevendans PA, Aengevaeren WR, Zwangina J, Birmond BI, van Rossum AC, Piek JJ, Zijlstra F; HEBE Investigators. Intramyocardial infusion of mononuclear bone marrow cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial. Eur Heart J 2011; 32: 1736-1747 [PMID: 21184540 DOI: 10.1093/eurheartj/ehq449]

Penicila M, Horak J, Kobylia P, Pytlík R, Kozák Z, Belohlávek O, Lang O, Skalicka H, Simek S, Paleček T, Detert L, Heurt A, Aschermann M, Widinsky P. Intramyocardial injection of autologous bone marrow-derived mononuclear cells in patients with large anterior acute myocardial infarction: a prematurely terminated randomized study. J Am Coll Cardiol 2007; 49: 2373-2374 [PMID: 17572255 DOI: 10.1016/j.jacc.2007.04.009]

Sürder D, Manka R, Lo Cicero V, Moccetti T, Rußbach K, Soncin S, Turchetto L, Radizzani M, Astori G, Schwitter J, Zuber M, Auf der Maur C, Jarashish P, Gaemperli O, Windecker S, Moschovitis A, Wulg A, Bühler I, Wys W, Kozker S, Landmesser U, Lüscher TE, Corti R. Intramyocardial injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. Circulation 2013; 127: 1968-1979 [PMID: 23596006 DOI: 10.1161/CIRCULATIONAHA.112.010335]

Karpov RS, Popov SV, Markov VA, Suslova TE, Ryabov VV, Poporina YS, Krylov AL, Sazonova SV. Autologous mononuclear bone marrow cell transplantation as regenerative technique after acute myocardial infarction. Bull Exp Biol Med 2005; 140: 640-643 [PMID: 16758644 DOI: 10.1007/s10517-006-0043-1]

Huikuri HV, Kerivinen K, Niemelä M, Ylitalo K, Säily M, Koistinen P, Savolainen ER, Ukkonen H, Pietilä M, Airaksinen JK, Knutti J, Mäkiäilio TH; FINCELL Investigators. Effects of intramyocardial injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 2008; 29:

Dorobantu M et al. Cell therapy end-points for ischemic disease

WJSC | www.wjgnet.com

215

December 26, 2017 | Volume 9 | Issue 12 |
with composite end points in cardiovascular studies: the story of major adverse cardiac events and percutaneous coronary intervention. J Am Coll Cardiol 2008; 51: 701-707 [DOI: 10.1016/j.jacc.2007.10.034]

Assmus B, Leistner DM, Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Sedding D, Yu J, Corti R, Matthey DG, Barth C, Mayer-WelATTERN C, Burch K, Sueselbeck T, Dill T, Hamm CW, Tonn T, Dimmeler S, Zeiher AM; REPAIR-AMI Study Group. Long-term clinical outcome after intra coronary application of bone marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival. Eur Heart J 2014; 35: 1275-1283 [DOI: 24569031 DOI: 10.1093/eurheartj/eho62]

Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. In: Martin-Rendon E, ed. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley Sons, Ltd, 2016 [DOI: 10.1002/14651858.CD007888.pub3]

Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Cochrane Corner: stem cell therapy for chronic ischaemic heart disease and congestive heart failure. Heart 2017; pii: heartjnl-2017-311684 [DOI: 10.1093/heartjnl-2017-311684]

Khan AR, Farid TA, Pathan A, Tripathi A, Ghaflighi S, Wysoczynski M, Bolli R. Impact of Cell Therapy on Myocardial Perfusion and Cardiovascular Outcomes in Patients With Angina Refractory to Medical Therapy: A Systematic Review and Meta-Analysis. Circ Res 2016; 118: 984-993 [PMID: 26838794 DOI: 10.1161/CIRCRESAHA.115.308056]

Creemers EE, Tijssen AJ, Pinto YM. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? Circ Res 2012; 110: 483-495 [PMID: 23230755 DOI: 10.1161/CIRCRESAHA.111.247452]

Kränkel N, Blankenberg S, Zeller T. Early detection of myocardial infarction-microRNAs right at the time? Ann Transl Med 2016; 4: 502 [DOI: 28149864 DOI: 210357/2016.12.12]

Schulte C, Westermann D, Blankenberg S, Zeller T. Diagnostic and prognostic value of circulating microRNAs in heart failure with preserved and reduced ejection fraction. World J Cardiol 2015; 7: 843-860 [PMID: 26730290 DOI: 10.4330/wjc.v7.i12.843]

Karakaş M, Schulte C, Appelbaum S, Ogda F, Lackner KJ, Müntel T, Schnabel RB, Blankenberg S, Zeller T. Circulating microRNAs strongly predict cardiovascular death in patients with coronary artery disease-results from the large AtheroGene study. Eur Heart J 2017; 38: 516-523 [PMID: 27357355 DOI: 10.1093/eurheartj/ehw250]

Jakob P, Kapcrowski T, Bränd-Schuracher S, Heg D, Klingenberg R, Stähl BE, Jaguszewski M, Rodondi N, Nančen D, Räber L, Vogt P, Mach F, Windecker S, Völker U, Matter CM, Lüscher TF, Landmesser U. Profiling and validation of circulating microRNAs for cardiovascular events in patients presenting with ST-segment elevation myocardial infarction. Eur Heart J 2017; 38: 511-515 [PMID: 28011706 DOI: 10.1093/eurheartj/ehw563]

Thum T, Cordelleri G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. Circ Res 2015; 116: 751-762 [PMID: 25677521 DOI: 10.1161/CIRCRESAHA.116.305349]

Kumarswamy R, Bauters C, Volkman I, Maury F, Fetsich J, Holzmann A, Lemesle G, de Groote P, Pinet F, Thum T. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. Circ Res 2014; 114: 1569-1575 [PMID: 24663402 DOI: 10.1161/CIRCRESAHA.114.309135]

Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. Circ Res 2014; 115: 668-677 [PMID: 25035510 DOI: 10.1161/CIRCRESAHA.115.303836]

Fish KM, Hajjar RJ. Mesenchymal Stem Cells &amp; Endothelial Function. ElBioMedicine 2015; 2: 376-377 [PMID: 26137582 DOI: 10.1016/j.embio.2015.04.015]

Stark DD, Weissleder R, Elizondo G, Hahn PF, Saini S, Todd LE, Wittenberg J, Ferrucci JT. Superparamagnetic iron oxide: clinical application as a contrast agent for MR imaging of the liver. Radiology 1988; 168: 297-301 [PMID: 3393649 DOI: 10.1148/radiology.168.2.3393649]
I, Bartunek J, Vanderheyden M, Kim HS, Kang HJ, Strauer BE, Lipinski MJ
10.1001/archinte.167.10.989
Arch Intern Med 2007; 167: 989-997 [PMID: 17533201 DOI: 10.1001/archinte.167.10.989]

Kostura L, Kraitchman DL, Mackay AM, Pittenger MF, Bulte JW. Feridex labeling of mesenchymal stem cells inhibits chondrogenesis but not adipogenesis or osteogenesis. NMR Biomed 2005; 18: 533-559 [PMID: 16229060 DOI: 10.1002/nbm.991]

Arbab AS, Yocum GT, Rad AM, Khakoo AY, Fellowes V, Read EF, Frank JA. Labeling of cells with ferumoxides-protonate sulfate complexes does not inhibit function or differentiation capacity of hematopoietic or mesenchymal stem cells. NMR Biomed 2005; 18: 533-559 [PMID: 16229060 DOI: 10.1002/nbm.991]

Penicka M, Widmisky P, Kobylinka P, Kozak T, Lang O. Images in cardiovascular medicine. Early tissue distribution of bone marrow mononuclear cells after transcoronary transplantation in a patient with acute myocardial infarction. Circulation 2005; 112: e63-e65 [PMID: 16043651 DOI: 10.1161/CIRCULATIONAHA.104.496133]

Vrtovec B, Poglajen G, Lezaic L, Sever M, Socan A, Donomavic D, Cernele P, Toerre-Amigone G, Haddad F, Wu JC. Comparison of intracoronary and intracardiac CD34+ cell transplantation in patients with nonischemic dilated cardiomyopathy. Circulation 2013; 128: S42-S49 [PMID: 24030420 DOI: 10.1161/CIRCULATIONAHA.112.002390]

Schächinger V, Aicher A, Döbert N, Röver R, Diener J, Fichtlscherer S, Assmus B, Seeger FH, Menzel C, Brenner W, Dinnmiller S, Zehir AM. Pilot trial on determinants of progenitor cell recruitment to the infarcted human myocardium. Circulation 2008; 118: 1425-1432 [PMID: 18794392 DOI: 10.1161/CIRCULATIONAHA.108.777110]

Yaghoubi SS, Jensen MC, Satyamurthy N, Budhiraja S, Paik D, Czemins J, Gambhir SS. Noninvasive detection of therapeutic cytotoxic T cells with 18F-FHBG PET in a patient with glioma. Chin Med J 2009; 122: doi: 10.1161/CIRCULATIONAHA.112.000230

Wu JC, Inabushi M, Sundaresan G, Schelbert HR, Gambhir SS. Positron emission tomography imaging of cardiac reporter gene expression in living rats. Circulation 2002; 106: 180-183 [PMID: 12105155 DOI: 10.1161/01.CIR.0000023620.9633.58]

Chen L, Tong JY, Kin H, Ren XM, Jin H, Wang QJ, Ma GS. Long-term effects of bone marrow-derived cells transplantation in patients with acute myocardial infarction: a meta-analysis. Chin Med J (Eng) 2013; 126: 353-360 [PMID: 23324289]

Jeong H, Yim HW, Cho Y, Park HJ, Jeong S, Kim HB, Hong W, Kim H. The effect of rigorous study design in the research of autologous bone marrow-derived mononuclear cell transfer in patients with acute myocardial infarction. Stem Cell Res Ther 2013; 4: 82 [PMID: 23849557 DOI: 10.1186/scrt233]

Dorobantu M et al. Cell therapy end-points for ischemic disease
Wen Y, Meng L, Xie J, Ouyang J. Direct autologous bone marrow-derived stem cell transplantation for ischemic heart disease: a meta-analysis. Expert Opin Biol Ther 2011; 11: 559-567 [PMID: 21388335 DOI: 10.1517/14712598.2011.560567]

Zhao Q, Ye X. Additive value of adult bone-marrow-derived cell transplantation to conventional revascularization in chronic ischemic heart disease: a systemic review and meta-analysis. Expert Opin Biol Ther 2011; 11: 1569-1579 [PMID: 21981749 DOI: 10.1517/14712598.2011.616491]

Donndorf P, Kundt G, Kaminski A, Yerebakan C, Liebold A, Steinhoff G, Glass A. Intramyocardial bone marrow stem cell transplantation during coronary artery bypass surgery: a meta-analysis. J Thorac Cardiovasc Surg 2011; 142: 911-920 [PMID: 21376346 DOI: 10.1016/j.jtcvs.2010.12.013]

Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. Circulation 2012; 126: 551-568 [PMID: 22730444 DOI: 10.1161/CIRCULATIONAHA.111.086674]

Jiang M, He B, Zhang Q, Ge H, Zhang MH, Han ZH, Liu JP, Li JH, Zhang Q, Li HB, Jin Y, He Q, Gong XR, Yin XY. Randomized controlled trials on the therapeutic effects of adult progenitor cells for myocardial infarction: meta-analysis. Expert Opin Biol Ther 2010; 10: 667-680 [PMID: 20384520 DOI: 10.1517/14712591003716437]

Cheng K, Wu F, Cao F. Intramyocardial autologous cell engraftment in patients with ischemic heart failure: a meta-analysis of randomised controlled trials. Heart Lung Circ 2013; 22: 887-894 [PMID: 23806195 DOI: 10.1016/j.hlc.2013.04.112]

Kandala J, Upadhyay GA, Pokushalov E, Wu S, Drachman DE, Singh JP. Meta-analysis of stem cell therapy in chronic ischemic cardiomyopathy. Am J Cardiol 2013; 112: 217-225 [PMID: 23623290 DOI: 10.1016/j.amjcard.2013.03.021]

Sadat K, Ather S, Aljaroudi W, Heo J, Iskandrian AE, Hage FG. The effect of bone marrow mononuclear stem cell therapy on left ventricular function and myocardial perfusion. J Nucl Cardiol 2014; 21: 351-367 [PMID: 24797128 DOI: 10.1007/s12350-013-9846-4]

Xu R, Ding S, Zhao Y, Pu J, He B. Autologous transplantation of bone marrow/blood-derived cells for chronic ischemic heart disease: a systematic review and meta-analysis. Can J Cardiol 2014; 30: 1370-1377 [PMID: 24726692 DOI: 10.1016/j.cjca.2014.01.013]

Tian T, Chen B, Xiao Y, Yang K, Zhou X. Intramyocardial autologous bone marrow cell transplantation for ischemic heart disease: a systematic review and meta-analysis of randomized controlled trials. Atherosclerosis 2014; 233: 485-492 [PMID: 24530783 DOI: 10.1016/j.atherosclerosis.2014.01.027]

Fisher SA, Brunskill SJ, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. Cochrane Database Syst Rev 2014; (4): CD007888 [PMID: 24777540 DOI: 10.1002/14651858.CD007888.pub2]

Martin-Rendon E. Meta-Analyses of Human Cell-Based Cardiac Regeneration Therapies: What Can Systematic Reviews Tell Us About Cell Therapies for Ischemic Heart Disease? Circ Res 2016; 118: 1264-1272 [PMID: 27081109 DOI: 10.1161/CIRCRESAHA.115.307540]

P-Reviewer: Kiselev SL, Liu L, Liu SH S-Editor: Ji FF L-Editor: A E-Editor: Lu YJ
