

# From bone marrow to the arterial wall: the ongoing tale of endothelial progenitor cells

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Several physiological and pathophysiological stimuli or drugs modulate endothelial progenitor cell (EPC) mobilization. Moreover, levels of circulating EPCs predict cardiovascular risk and left ventricular remodelling after myocardial infarction. Nevertheless, our understanding in this field is complicated by lack of an unequivocal definition of EPCs, thus limiting their clinical applications. This review summarizes current knowledge and uncertainties on EPC characterization and mobilization in the attempt to define their role in the management of cardiovascular diseases.

## Keywords

Endothelial progenitor cells • Bone marrow-derived stem cells • Acute myocardial infarction • Stem cell mobilization

## Introduction

Almost a century ago, Cohnheim<sup>1</sup> proposed that the bone marrow is a reservoir of stem cells capable of regenerating not only the bone marrow itself but also solid organs. Although the bone marrow remains far from being the source of eternal youth, after the identification of putative circulating endothelial progenitor cell (EPC),<sup>2</sup> almost all tissues have been generated, at least *in vitro*, from bone marrow cells<sup>3–7</sup> and we are witnessing a hot debate on which stem cells have the best potential to regenerate the damaged heart. We believe that the vast experience acquired in several years by haematologists should be the ideal platform for developing the future of stem cells in cardiology.

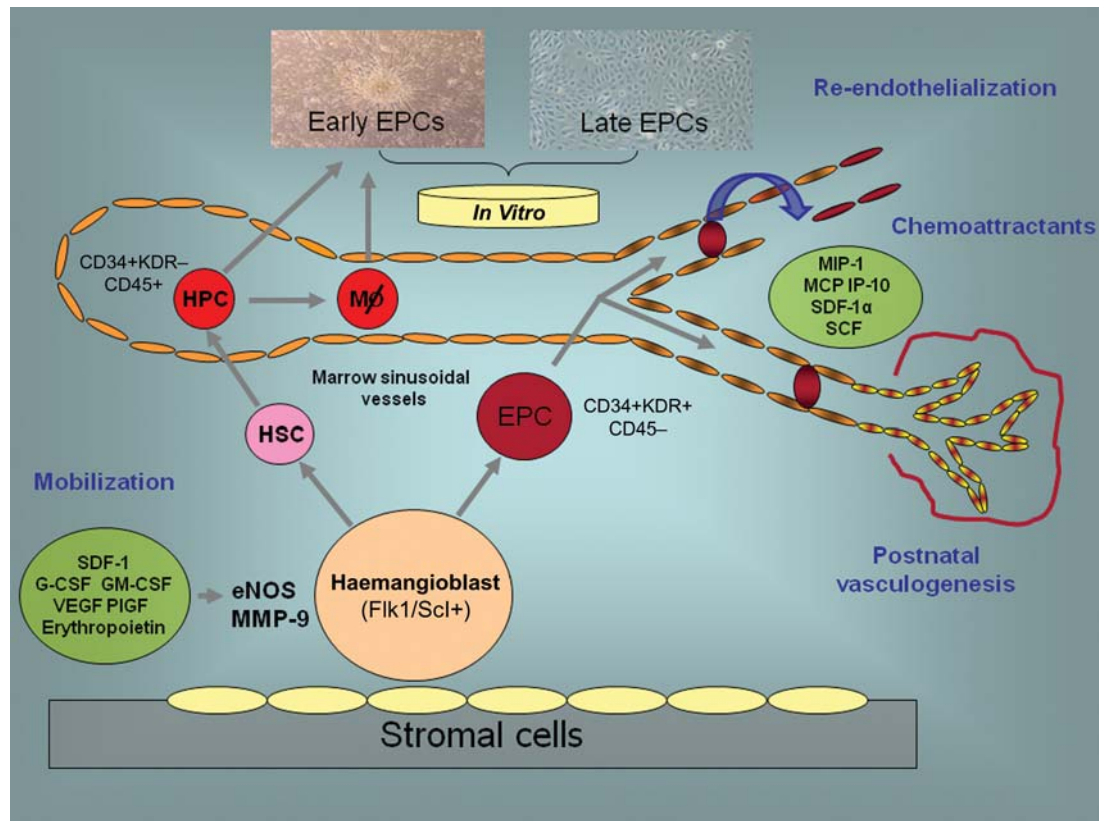
Bone marrow is constituted by different types of stem/progenitor cells, including—but not limited to—the multipotent adult progenitor cells, able to regenerate several tissue layers, mesenchymal stem cells, and haemangioblasts, the common putative precursors of haematopoietic and the endothelial lineages.<sup>8</sup> The haematopoietic stem cells, with unlimited capacity of self-renewal and differentiation, are the thousandth part of a mixed cell population identified by the surface expression of the CD34 epitope. The remaining CD34+ cells are progenitor cells with limited capacity of bone marrow regeneration, and among them, EPCs have been identified according to several different functional and phenotypic criteria.

Even though the exact mechanisms of neoangiogenesis and neo-vascularization are still poorly understood, vessels could be repaired not exclusively by adjacent mature and terminally differentiated endothelial cells, but also by the bone marrow-derived EPCs. These cells would reside in the bone marrow and from there would be repeatedly mobilized in response to several stimuli peripheral factors (Figure 1).<sup>9</sup> The principal mechanism of EPC mobilization from bone marrow seems to depend on the activation of endothelial nitric oxide synthase (eNOS) in the presence of several mobilizing factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PIGF).<sup>10</sup>

Vascular injury in different models of myocardial or peripheral limb ischaemia has been shown to effectively mobilize circulating EPCs, which localize at the site of damage where they divide, proliferate, and adhere to the sub-endothelium promoting growth of new vascular endothelium.<sup>11</sup> In particular, Stellos and Gawaz<sup>12</sup> showed in a mouse model that after endothelial injury, the platelet adhesion to the vascular wall induces a cytokine-mediated release of stromal-derived factor-1 (SDF-1) which, in turn, determines recruitment and proliferation of CD34+ CXCR4+ VEGFR1+ (flt-1+) EPCs and constitutes the major determinant of re-endothelization together with a possible paracrine effect of EPCs on endothelial cells, mediated by angiogenic factors, that could stimulate the proliferation of adjacent endothelial cells.

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**Figure 1** Mechanism and mediators of EPC mobilization. Adult bone marrow contains the haemangioblast, the putative common precursor for haematopoietic, and endothelial lineage. These cells are mobilized by several factors mainly through NO and MMP-9-mediated mechanisms, share common antigens, like the CD34, and differ for the expression of CD45, the common leucocyte antigen that lacks on true circulating endothelial progenitor cells. Nevertheless, cells from haematopoietic lineage also can form colonies of early EPCs *in vitro*. Colonies of late EPCs *in vitro* are generated from 'true' circulating EPCs (CD34+, KDR+, and CD45-). Circulating EPCs can participate to re-endothelialization, or possibly to post-natal vasculogenesis, directly or through a paracrine effect (see text for details).

Although it is tempting to postulate that the reparative release of EPCs could compensate for the simultaneous shedding of endothelial cells from the vascular wall, no study, to the best of our knowledge, has so far demonstrated a clear correlation between vascular damage (assessed by measuring circulating endothelial cells) and repair (assessed by measuring circulating EPCs).<sup>13</sup> Furthermore, recently in an elegant experimental model of parabiosis, no contribution to endothelial repair or to tumour growth was observed from putative bone marrow-derived endothelial cells, raising doubts about the relevance of this reparative mechanism.<sup>14</sup> Similarly, a recent paper of Rodriguez-Menocal *et al.*<sup>15</sup> showed that the contribution of bone marrow-derived progenitor cells to neo-intimal proliferation in a rat balloon injury model was very limited.

## Characterization of endothelial progenitor cells

The term 'endothelial progenitor cell' was used to define many different cell populations. In general, it can be referred to certain

cells obtained from culture of mononuclear cells and to circulating cells characterized by FACS analysis.

## Cultured endothelial progenitor cells

Several techniques were developed to plate peripheral blood mononuclear cells to give rise to EPC colonies.<sup>16</sup> Two major cell types were demonstrated to originate from these assays, the so-called early-outgrowth EPCs and late-outgrowth EPCs, according to their time-dependent appearance in culture, and to other features, such as morphology, proliferation rate, gene expression profile, and functional properties.<sup>17,18</sup> The vast majority of studies, including the pivotal studies by Asahara *et al.*<sup>2</sup> and by Hill *et al.*,<sup>19</sup> used early-outgrowth EPCs [also known as endothelial cells-like (ECs-like), colony-forming unit (CFU) of ECs, circulating angiogenic cells, attaching cells, early-outgrowth culture expanded EPCs, culture-modified mononuclear cells, and early EPCs]; they are spindle-shaped cells obtained by culturing isolated mononuclear cells for 4–7 days. Classically, their number is evaluated at 7th day,<sup>2</sup> but they can last up to 4 weeks. Phenotypically, these cells share several characteristics with mature endothelial cells: they take up acetylated LDL, bind to *Ulex europaeus*

**Table 1** Factors affecting EPC mobilization

Stimulus	Response
Age <sup>36–38</sup>	↓ EPC cytopoiesis ↓ EPC mobilization (chronic e acute) ↓ EPC survival ↓ EPC functional activity
Oestrogens <sup>35</sup>	↑ EPC concentration
Exercise <sup>31–34</sup>	↑ EPC concentration
CV risk factors	
Number of CV risk factors <sup>42</sup>	↓ EPC number
Framingham CV total risk score <sup>45</sup>	↓ EPC number ↓ CD34/KDR+ number
Optimal flow-mediated dilation <sup>45</sup>	↑ EPC number ↑ CD34/KDR+ number
Smoking <sup>44</sup>	↓ EPC number
Hypertension <sup>46</sup>	↑ EPC proliferation ↓ EPC survival
Hypercholesterolaemia <sup>47</sup>	↓ EPC proliferation ↓ EPC migratory capacity ↓ EPC vasculogenetic property ↓ EPC survival
Diabetes mellitus <sup>48</sup>	↓ EPC number
Myocardial ischaemia	
Myocardial infarction <sup>56–58</sup>	↑ EPC number ↑ CD34+ cell number
Unstable angina <sup>61</sup>	↑ EPC number
Stress test-induced ischaemia in CSA <sup>62</sup>	↑ CD34/KDR+ number
Severe CAD without ischaemia <sup>67</sup>	↓ EPC number
Severe CAD requiring revascularization <sup>68</sup>	↑ EPC number
Global ischaemia in extracorporeal circulation <sup>63</sup>	↑ CD34+ cell number
Cardiac syndrome X <sup>64,65</sup>	↑ EPC number ↓ e-CFU capacity ↓ EPC functional activity
Myocardial necrosis	
Trans-catheter ablation <sup>60</sup>	↑ EPC number
Heart failure	
Early NYHA class (I and II) <sup>53</sup>	↑ CD34/KDR+ number
Advanced NYHA class (III and IV) <sup>53</sup>	↓ CD34/KDR+ number
Post-ischaemic heart failure <sup>70</sup>	↓ e-CFU capacity ↓ EPC niches in bone marrow
Primary angioplasty <sup>57–59</sup>	↑ CD34+ cell number ↓ EPC number
Renal failure <sup>75</sup>	↓ EPC number

agglutinin, and express CD31, CD34 (generally at low levels), VE-cadherin, VEGFR-2, and von Willebrand factor. Differently from mature endothelial cells, early-outgrowth EPCs share some similarities with monocytes because they express the monocytic

marker CD14<sup>20</sup> and the panleucocytic marker CD45.<sup>21</sup> These features make the origin of early-outgrowth EPCs from haematopoietic lineage very likely. Moreover, early-outgrowth EPCs have a limited proliferative capacity; do not form directly a vascular network *in vitro* but they can contribute to its formation by a paracrine mechanism.<sup>22,23</sup> In contrast, late-outgrowth EPCs start proliferating only after 2–3 weeks in culture.<sup>24</sup> They show cobblestone morphology and are relatively rare. These cells, also called blood-derived outgrowth endothelial cells<sup>25</sup> or endothelial colony-forming cells,<sup>26</sup> are capable of up to 20 population doublings and can form a vascular network. In addition to CD34, they express endothelial markers, like KDR, CD146, and VE-cadherin, whereas they do not express haematopoietic markers, like CD45 and CD14, and consequently their progeny better resemble mature endothelial cells. In conclusion, late-outgrowth EPCs are probably more related to replacement of defective endothelial cells and vasculogenesis, and early-outgrowth EPCs may have a role as a biomarker, especially considering the amount of data available on their prognostic value that will be discussed later in the present review.

Circulating endothelial progenitor cells

To perform pathophysiological human studies, a simpler and more pragmatic approach has been considered to count circulating cells expressing surface markers which could be prototypical for the EPC phenotype by means of flow cytometric analysis. These cells can be collected from bone marrow or peripheral blood, in particular from whole blood or after separation of the mononuclear cells by Ficoll®. Typical markers of EPCs are CD34, which was originally used in the paper of Asahara et al.,<sup>2</sup> the VEGF receptor 2, also known as kinase-insert domain receptor (KDR), and CD133.<sup>27</sup> However, not all these markers are expressed together. Indeed, in the transition from bone marrow towards the blood stream EPCs would lose CD133 and, more slowly, CD34, while they end up acquiring new markers, like CD146 but not the CD14, which remains a feature of monocytic cells.<sup>18</sup> According to this view, early circulating EPCs could be recognized by the coexpression of CD34, CD133, and KDR. Nevertheless, recently it was demonstrated that this population is represented by enriched CD45+ haematopoietic precursors. Considering that CD45 antigen marks the haematopoietic lineage from foetal life to adulthood and is absent in mature endothelial cells and that late-outgrowth EPCs appear to derive from ‘true’ circulating EPCs, the latter are more likely contained within the CD45-subset of the CD34+ KDR+ cells.<sup>16</sup> In conclusion, a broader definition of circulating EPCs, which includes cells able to give origin to both early and late-outgrowth EPCs, is probably represented by the CD34+ KDR+ cells. The subset of these cells, not expressing the CD45 antigen, however, probably better identifies true circulating EPCs. All these considerations should be born in mind to correctly interpret results from clinical studies that used different combination of markers<sup>28</sup> and that will be presented in the next parts of the present review. Indeed, considering that this subpopulation could include also shed endothelial cells, the research for a complete unequivocal definition of circulating EPCs by means of flow cytometry continues.

## Physiological and pathophysiological stimuli of endothelial progenitor cell mobilization

Endothelial progenitor cell release from the bone marrow is mediated by eNOS-derived nitric oxide (NO) produced by the regulatory components of bone marrow microenvironment, i.e. osteoblasts and endothelial cells. Accordingly, substances that increase NO bioavailability, like growth hormone (GH) and insulin growth factor-1 (IGF-1), increase EPC levels.<sup>29</sup> In contrast, higher levels of endogenous substances that impair NO bioavailability, like asymmetric dimethylarginine (ADMA), are associated with lower levels of EPCs and with *in vitro* inhibition of: (i) mobilization and differentiation of EPCs, (ii) incorporation into endothelial tube-like structures, and (iii) formation of CFUs from cultured peripheral blood mononuclear cells.<sup>30</sup>

Physiological factors mobilizing EPCs from bone marrow niches include physical exercise, which acts through an NO- and VEGF-mediated mechanism (Table 1).<sup>31–34</sup> Oestrogens mobilize EPCs through a direct action on  $\alpha$  and  $\beta$  oestrogen receptors, via matrix metalloproteinase-9 (MMP-9)- and eNOS-mediated mechanism, helping to explain the lower rate of cardiovascular events in pre-menopausal women when compared with men.<sup>35</sup>

In contrast, several studies have demonstrated that ageing has a negative impact on EPC at different steps.<sup>36,37</sup> In fact, middle-aged and elderly subjects compared with young subjects have significantly less EPCs, with an impaired function. This could possibly be related to an ageing-dependent reduction in IGF-1 levels. Indeed, treatment with GH restores normal EPC levels and reduces their senescence through an increase in IGF-1.<sup>38</sup> A reduction in the mechanisms of vascular repair by EPCs could also be related to ageing-dependent decrease in plasma concentration of VEGF which could limit EPC mobilization and differentiation.<sup>39</sup> Of note, ageing blunts bone marrow response to pathophysiological stimuli.<sup>40</sup> Thus, a senescent and less competent bone marrow might be unable to release a critical mass of EPCs in critical conditions.<sup>41</sup>

With regard to pathophysiological stimuli, the EPC number was proven to be inversely related to the number of cardiovascular risk factors<sup>42</sup> and to the Framingham cardiovascular total risk score, and directly related to brachial reactivity (Table 1).<sup>19</sup> In general, the greater the EPC number, the better is vasculature health. Interestingly, EPC count was proven to be more predictive of brachial flow-mediated dilation than cardiovascular risk factors burden, thus suggesting that EPCs can efficiently counteract the detrimental effect of risk factors on vascular function.<sup>19</sup> In contrast, this finding was not in a large population-based study of Xiao *et al.*,<sup>104</sup> who found that an increased Framingham risk score was strongly associated with higher levels of EPCs, despite a mild but significant inverse correlation with the extent of coronary atherosclerosis.

Among suppressive factors, smoking increases oxidative stress and reduces NO bioavailability resulting in depletion of EPCs for vascular repair in a dose-dependent manner<sup>43</sup> and with a rapid amelioration after smoking cessation.<sup>44</sup> Yet, in a large study by

Werner *et al.*,<sup>45</sup> prevalence of smokers was higher among subjects with the higher levels of EPCs.

In arterial hypertension, angiotensin (AT) II accelerates EPC senescence by reducing telomerase activity and provoking EPC oxidative stress, although it also stimulates VEGF-mediated EPC proliferation, probably due to KDR upregulation.<sup>46</sup>

Hypercholesterolaemia determines a reduction in EPCs' proliferative, migratory, and vasculogenetic properties,<sup>47</sup> secondary to a rise in senescence/apoptosis ratio, as demonstrated after EPC incubation with LDL-oxidized, whereas HDL-cholesterol could exert a vascular protection increasing EPC number.

Diabetes mellitus plays a pivotal role in the modulation of EPC mobilization and function (Table 1). In diabetics, EPC levels are strictly related to glycaemia levels, and interestingly to the ankle-brachial index.<sup>48</sup> Among the different complications of diabetes, vascular complications are those mostly associated with reduced peripheral number of EPCs, thus suggesting that EPC depletion can be involved in their pathogenesis.<sup>48</sup> A severely impaired re-endothelialization capacity of EPCs in diabetics might be due, at least in part, to an increased NADPH oxidase-dependent superoxide production and subsequently reduced NO bioavailability.<sup>49</sup> However, activation of NADPH oxidase could be less important compared with uncoupling of the eNOS, resulting in superoxide anion formation instead of NO that Thum *et al.*<sup>50</sup> observed in EPCs from diabetics. This is due to a reduced number and to functionally impaired EPCs, likely contributing to the pathogenesis of vascular disease in diabetes.

C-reactive protein has a direct role in the reduction of EPC number and activity, influencing adhesion through a reduction of mRNA transcription of chemoattractant factors as monocyte chemoattractant protein-1 and -2, macrophage inflammatory protein-1 $\alpha$ , colony-stimulating factor, and IFN- $\gamma$  inducible protein-10.<sup>51,52</sup> C-reactive protein also mediates suppressor of cytokine signalling upregulation involved in JAK-STAT pathway inhibition, which plays a pivotal role in EPC proliferation and growth. Similarly, TNF $\alpha$ , with its well-known myelosuppressive effect, could be responsible for the reduction of haemopoiesis and EPCs levels observed in the late phases of heart failure.<sup>53</sup>

Acute myocardial infarction (AMI) is the most established acute pathological stimulus for EPC mobilization (Table 1). After an AMI, progenitor/stem cells are mobilized from bone marrow, released into peripheral blood, and subsequently homed in the myocardium.<sup>54,55</sup> The relation between EPC mobilization and cardiac repair has extensively been studied. In 2001, Shintani *et al.*<sup>56</sup> increased the concentration of cultured EPCs and of peripheral CD34+ cells after AMI peaking at 7th day. These findings were substantially confirmed by other groups including ours,<sup>57–59</sup> despite time to peak varied in the different studies, ranging from 5 to >7 days, largely dependent from patient selection criteria and timing and modalities of reperfusion. In particular, we found a higher concentration of CD34+ cells in patients with an AMI than in those with chronic stable angina and in healthy subjects. At follow-up, EPC levels fell to values similar to those found in control healthy subjects. Among patients with AMI, the number of progenitor cells ranged from >20 to <1 CD34+ cells/ $\mu$ L, indicating the presence of good and poor mobilizers. The degree of spontaneous EPC mobilization was predicted by statin therapy,



anterior localization of AMI, probably for the greater extent of ischaemic tissue, and by primary angioplasty to re-vascularize the infarct-related artery, possibly for the prompt and large release of mobilizing factors into peripheral circulation after coronary recanalization. In contrast, a smaller clinical study by Müller-Ehmsen et al.<sup>59</sup> failed to demonstrate a higher concentration of EPCs in patients who had undergone primary angioplasty, suggesting that inclusion criteria and timing and methods for EPC evaluation could be cause of relevant differences among different studies.

Post-AMI EPC mobilization could depend both from prolonged ischaemia and/or from myocardial necrosis. Although one study demonstrated that non-ischaemic myocardial necrosis such as catheter-based radiofrequency ablation can increase circulating EPCs,<sup>60</sup> the vast majority of data supports the notion that the pivotal role is played by myocardial ischaemia. For instance, we could not find any correlation between EPC mobilization after AMI and release of markers of myonecrosis, whereas the amount of mobilized CD34+ cells was predicted by a larger ischaemic territory.<sup>57</sup> Moreover, patients with unstable angina and no evidence of myonecrosis were found to have increased EPC levels compared with patients with stable angina, with a similar extent of coronary artery disease (CAD).<sup>61</sup> Furthermore, in stable patients with known CAD, exercise stress testing is followed by mobilization of CD34+/KDR+ cells peaking at 24–48 h.<sup>62</sup> Similarly, in the model of global ischaemia induced by extracorporeal circulation during coronary artery bypass grafting, an increase in concentration of CD34+ cells was demonstrated, independently from clinical characteristics.<sup>63</sup> Finally, in patients with cardiac syndrome X, a clinical model of microvascular coronary dysfunction characterized by angina and evidence of stress-induced myocardial ischaemia, a significant increase in circulating EPCs was demonstrated compared with matched control subjects,<sup>64</sup> associated with lower functional capacities.<sup>65</sup>

A rapid recruitment of EPCs from bone marrow, peaking 3 days later, has been demonstrated following repeated ischaemia inducing preconditioning. This phenomenon could participate in myocardial protection from the ischaemic damage, at least in the experimental model. In fact, in animals in which an ischaemic preconditioning was induced, the increase in EPC levels was associated with a reduction in the infarcted area in comparison to those not treated with preconditioning and in which, consequently, no EPC mobilization was shown.<sup>66</sup>

Conflicting data on EPC mobilization exist in patients with CAD. Considering that risk factors are inversely related to EPC levels and patients with CAD, on average, have a high risk profile, patients with severe CAD should, theoretically, have less EPCs. This notion is supported by the results of a recent study showing that patients with multivessel disease had significantly lower levels of EPCs when compared with patients with single-vessel disease or without CAD.<sup>67</sup> In contrast, in a previous study, Güven et al.<sup>68</sup> found that the number of EPCs was directly related to the extent of coronary atherosclerosis. These discrepancies could be explained by significant differences in the ischaemic burden. In fact, in the study of Güven et al., patients who require coronary revascularization had significantly higher levels of EPCs when compared with patients without indication for revascularization. A lower EPC count in patients with CAD could be caused by increased consumption, impaired mobilization

from the bone marrow, or both. Thum et al. found that the severity of CAD was significantly correlated to plasma concentrations of ADMA, a potent endogenous inhibitor of NO synthase. Levels of ADMA were demonstrated inversely related to the number of CD34+/CD133+ cells and of endothelial colony-forming units (e-CFUs), whereas *in vitro* ADMA was able to impair formation of e-CFUs and EPC differentiation. Consequently, the interaction between ADMA and EPCs could contribute to cardiovascular risk and may help explaining low numbers and function of EPCs in patients with CAD.<sup>30</sup>

The relation between heart failure and EPC levels is complex (Table 1); Valgimigli et al.<sup>53</sup> demonstrated that functional NYHA class is related to different levels of CD34+ cells. Indeed, compared with healthy control subjects, patients in the lower functional classes (NYHA I and II) had higher levels of CD34+ cells, which, in contrast, were significantly lower in NYHA III and IV patients. These lower levels of EPCs were interpreted to be related to the higher serum levels of TNF $\alpha$ , a known myelosuppressive cytokine. In contrast, in a large study in patients with ischaemic or non-ischaemic heart failure, Michowitz et al.<sup>69</sup> found a mild direct correlation between number of e-CFUs and NYHA class. Recently, Kissel et al. provided novel evidence in patients with post-ischaemic heart failure of a selective functional exhaustion of the EPCs. Interestingly, bone marrow niches as well as the colony-forming capacity seemed to be reduced, whereas bone marrow haematopoietic progenitor cell number was preserved.<sup>70</sup> This could be related to a reduction of the bio-availability of the NO in the bone marrow niches, as the NO has a pivotal role in modulating the activity of MMP-9 that is required for the mobilization of EPC but also for the transfer of endothelial cell to a proliferative niche.<sup>70</sup>

## Mediators of endothelial progenitor cell mobilization and homing

Several mobilizing factors, such as granulocyte colony-stimulating factor (G-CSF), granulocyte monocyte colony-stimulating factor, SDF-1 and VEGF, and erythropoietin (Epo), via AKT protein kinase pathway activation, were demonstrated to mediate EPC mobilization, proliferation, and migration.

In particular, we found that G-CSF, VEGF, and SDF-1 $\alpha$  serum levels were significantly higher in patients with AMI, compared with chronic stable angina patients and to controls.<sup>71</sup> More interestingly in patients with AMI, CD34+ cell count was significantly related to G-CSF levels, suggesting that they could be responsible for the phasic EPCs mobilization in this setting. Moreover, in a similar clinical setting, Shintani et al.<sup>56</sup> found that the amount of mobilized CD34+ cells was significantly correlated to VEGF levels.

Of note, the receptor for Epo, whose main function is to stimulate the proliferation of early erythroid precursors and the differentiation of later precursors of the erythroid lineage, is also on endothelial cells, suggesting again a common ontogenesis for haematopoietic and endothelial lineage. Erythropoietin was proven to increase the proliferative and adhesive properties of EPC *in vitro*,<sup>72</sup> the number of circulating EPCs in experimental models *in vivo*,<sup>73</sup> and of CD34+/CD45+ cells in humans.<sup>74</sup> These findings suggest

that lower levels of EPCs in conditions associated with lower levels of Epo, like in chronic renal failure, might contribute to its unfavourable prognosis.<sup>75</sup>

The most important role in tissue engraftment is played by the local concentration of SDF-1 $\alpha$  and its cell receptor CXCR-4. The importance of the expression of SDF-1 $\alpha$  for the homing of progenitor cells in the heart in the early phases of myocardial infarction<sup>76</sup> and in the ischaemic muscle after experimental hindlimb ischaemia<sup>77</sup> has been clearly demonstrated in experimental models. Yamaguchi *et al.*<sup>78</sup> demonstrated that local concentration of SDF-1 $\alpha$  with its receptor on CXCR4 on EPCs was directly correlated to neovascularization. In addition, we found that after myocardial infarction, circulating CD34+ cells were characterized by an enhanced expression of CXCR4, the SDF-1 $\alpha$  receptor on their surface, suggesting a potential myocardial homing for these cells<sup>57</sup> and determining a surface phenotype reminiscent for the G-CSF mobilized CD34+ cells.<sup>79</sup>

## Effects of drugs on endothelial progenitor cell mobilization

Several drugs have been demonstrated to increase circulating EPC levels (Table 2). ACE-inhibitors are recommended in post-infarction patients as they reduce mortality and severity of unfavourable left

ventricular (LV) remodelling. Notably, ramipril<sup>80</sup> and enalapril<sup>81</sup> were shown to increase EPC levels both in the experimental model and in patients, probably interfering with the CD26/dipeptidylpeptidase IV system, which is a membrane-bound extracellular peptidase with the ability to cleave chemokines containing the essential N-terminal X-Pro or X-Ala motif, such as SDF-1 $\alpha$ /CXCL12, and which serves as a chemoattractant for human CD34+ cells and stem/progenitor cell populations. Similar findings were found with AT II inhibitors, like valsartan.<sup>82</sup> With regard to 3-hydroxy-3-methyl-glutaryl-CoA reductase-inhibitors, several more robust evidences suggest that they can influence EPC levels and function. Vasa *et al.*<sup>83</sup> first demonstrated that in patients with CAD, statins administrated for 4 weeks can increase up to threefold EPC number improving their functional activity. Moreover, an increase migratory capacity was able to accelerate re-endothelialization through a reduced senescence and an increased proliferation via the activation of cell cycle regulatory genes.<sup>84</sup> These effects were related, at least partially, to an increased activity of the telomere capping protein, which could prevent telomere shortening and DNA damage pathways resulting in an improved functional activity of EPCs. This is particularly interesting when considering that telomere length was demonstrated to be directly correlated to the number of CFUs in patients with ischaemic heart failure.<sup>85</sup> Finally, in the large study of Werner *et al.*,<sup>45</sup> a significantly higher prevalence of statin therapy was present in the group of patients with the highest tertile of EPCs.

In a small randomized clinical trial in which we randomized patients with ST segment elevation myocardial infarction treated with a primary or rescue PCI to an intensive (atorvastatin 80 mg from the admission up to 4 month) or to a standard statin treatment (atorvastatin 20 mg from the discharge up to 4 month), we found that during hospitalization there was no difference in EPC levels between the two different groups. Nevertheless, atorvastatin 80 mg was associated with significantly higher levels of EPCs at 4 months of follow-up when compared with patients randomized to standard treatment with atorvastatin 20 mg.<sup>86</sup> Despite a chronic and continuous treatment with statins could be associated with an exhaustion of the bone marrow pool of EPCs,<sup>87</sup> our findings, if confirmed, could provide a possible mechanistic explanation for the reduction of acute coronary events associated with intensive statin treatment when compared with standard treatment,<sup>88,89</sup> as mobilization of EPCs by statins could favour a more efficient 'healing' of the culprit stenosis. Interestingly, Thum *et al.*<sup>90</sup> attributed post-infarction EPC mobilization to the concomitant use of ACE-inhibitors and statins, rather than to the release of endogen mediators caused by ischaemia, proposing that in the early phases after myocardial infarction, biohumoral alterations in the bone-marrow would not favour, but rather impair EPC function and mobilization. Such alterations would determine a reduced extracellular signal-regulated kinase 1 and 2 phosphorylation activity, responsible for MMP-9 inhibition and an elevated ROS systemic levels, with a following reduced bone marrow stem cells mobilization.

Insulin was demonstrated to increase significantly CD34+ CD133+ cells in patients with type II diabetes mellitus. Interestingly, this effect was particularly apparent in patients with the -3' A/G variant of the SDF-1 $\alpha$  gene.<sup>91</sup> Moreover, insulin was demonstrated to increase the ability to form e-CFUs. This effect was due to the

**Table 2** Drugs affecting EPC mobilization

Drugs	Response
ACE-inhibitors <sup>80,81</sup>	↑ EPC number
AT II antagonists <sup>82</sup>	↑ EPC number
Statins <sup>83,84,86</sup>	↑ EPC number
PPAR $\gamma$ <sup>93-95</sup>	↑ EPC number ↑ EPC functional activity
Insulin <sup>91,92</sup>	↑ EPC number ↑ EPC clonogenic properties
Growth hormone <sup>29,38</sup>	↑ EPC number ↑ EPC proliferation ↑ EPC migration
IGF-1 <sup>29,38,92</sup>	↑ EPC number ↑ EPC differentiation ↑ EPC migratory capacity ↑ e-CFU
Nitroglycerin	↑ EPC number
Isosorbide-5-dinitrate <sup>96,97</sup>	↓ EPC migratory capacity
Pentaerythritol tetranitrate <sup>97</sup>	↑ EPC number ↑ EPC migratory capacity
Erythropoietin <sup>75</sup>	↑ EPC number ↑ EPC proliferation ↑ EPC adhesive properties
G-CSF <sup>71</sup>	↑ CD34+ cells ↑ EPC number

IGF-1 receptor signalling and not mediated by the insulin receptor.<sup>92</sup> Peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ )-agonists, like rosiglitazone, also were demonstrated to restore circulating levels and functional properties of EPCs, through a reduction of the NADPH oxidase activity, an increased NO availability, restoring *in vivo* re-endothelialization capacity of EPCs from diabetics. Interestingly, this favourable effect of rosiglitazone should be independent from the glycaemic control.<sup>93</sup> Moreover, another PPAR $\gamma$ -agonist, the pioglitazone, showed in mice the capacity to increase EPC number and their functional capacity by inhibition of EPC apoptosis in an NO-independent manner.<sup>94</sup> However, the beneficial effects of pioglitazone could be biphasic depending on the dose; indeed, a low dose of pioglitazone was demonstrated to improve *in vitro* EPC adhesion and differentiation finally resulting in an increased EPC number, whereas a higher dose resulted in a TGF- $\beta$ 1-mediated suppression of EPC development.<sup>95</sup>

Finally, considering that mobilization of progenitor cells is an NO-mediated phenomenon, it is surprising that little data were published on the effect of organic nitrates on EPCs. DiFabio et al.<sup>96</sup> demonstrated that similarly to the detrimental effects on endothelial function induced by increased vascular stress, nitroglycerin induces increased apoptosis and decreased phenotypic differentiation, migration, and mitochondrial dehydrogenase activity in EPCs, despite higher levels of circulating CD34+ cells. However, probably not all organic nitrates are similar: Thum et al. tested the effects of different organic nitrates in rats finding that both isosorbide-5-dinitrate (like nitroglycerin) and pentaerythritol tetranitrate (or its major metabolite pentaerythrityl trinitrate) were able to increase circulating EPC levels. Nevertheless, only EPCs from isosorbide-5-dinitrate-treated animals displayed impaired migratory capacity and increased reactive oxygen species formation. This effect could be dependent on the specific antioxidative capacity of pentaerythrityl trinitrate and possibly clinically relevant.<sup>97</sup>

## Clinical implications of endothelial progenitor cell mobilization

Taken together, the clinical implications of the large body of information acquired in the past 10 years on the physiology and pathophysiology of EPCs are profound, as peripheral levels of EPCs could mirror both vascular health and the potential for heart repair. These findings could explain atherosclerosis and myocardial damage in a new perspective that is complementary to the theory of 'reaction to injury'. In this new perspective, vascular damage is caused by loss of the balance between vascular and myocardial injury and EPC-mediated repair. Accordingly, the individual susceptibility to atherogenic stimuli causing endothelial dysfunction could be determined not only by the number and the duration of exposure to risk factors but also by the capability to promptly mobilize EPCs and repair endothelial and myocardial injury. Consequently, the compelling evidence suggesting a favourable role of EPCs in vascular and myocardial repair would support a prognostic impact of EPC mobilization in patients with CAD. With regard to this point, two interesting studies suggested that patients with CAD and high levels of EPCs have a significantly better prognosis

compared with patients with low levels.<sup>45,98</sup> In particular, Werner et al. demonstrated in 519 patients with CAD that increased levels of CD34+/KDR+ EPCs were associated with a reduced risk of death from cardiovascular causes (hazard ratio 0.31; 95% CI, 0.16–0.63;  $P = 0.001$ ) and of recurrence of revascularization (hazard ratio 0.77; 95% CI, 0.62–0.95;  $P = 0.002$ ) and hospitalization (hazard ratio 0.76; 95% CI, 0.63–0.94) but not of myocardial infarction or death from all causes. The prognostic impact of EPC levels was maintained also in a large study in patients with congestive heart failure, in which they were demonstrated to be the most important determinant of prognosis with the advanced age and the diabetes mellitus at a Cox proportional regression analysis.<sup>69</sup> Similar considerations can be applied to post-AMI patients; indeed, recent findings support the notion that in patients with myocardial infarction mobilization of EPCs could influence mechanisms of LV remodelling. We found a direct correlation between peripheral CD34+ cell count and changes of LV ejection fraction at follow-up and an inverse significant correlation between peripheral CD34+ cell count and changes of LV volumes and wall motion score index. This was particularly apparent in patients with persistently higher levels of CD34+ cells.<sup>57</sup> Taken together, these findings support the contribution of bone marrow to cardiac repair probably by improving microvasculature of the peri-infarct region. Subsequently, Wojakowski et al.<sup>99,100</sup> demonstrated that CD34+/CXCR4+ or CD34+/CD117+ cells mobilized from bone marrow after an AMI expressed early cardiac and endothelial superficial markers, suggesting their potential role as 'committed tissue cells' designed to improve cardiac function, as demonstrated by the significant correlation with post-infarction LV function.

On these bases, several clinical trials investigated the possibility to favourably affect post-infarction LV function through the pharmacological mobilization of progenitor cells using G-CSF. Nine randomized controlled studies evaluated the safety and efficacy profile of G-CSF in the setting of the acute/subacute phase of myocardial infarction in humans on a total of 409 patients.<sup>101</sup> G-CSF in general is well tolerated, but a meta-analysis failed to find a beneficial effect of G-CSF on LV function.<sup>102</sup> Yet, a beneficial effect was seen in the subset of patients with impaired baseline LV function.<sup>103</sup> Future properly powered randomized controlled studies ideally targeting patients with large anterior MI with poor response to early reperfusion are warranted to further clarify the role of G-CSF in this setting.

In conclusion, in the last few years, a large body of evidence has been accumulated suggesting the importance of EPCs in the mechanisms of vascular repair. Regardless of their therapeutic applications, the role of EPCs as a marker of vascular health and prognosis is already well established. More importantly, EPC might not be simply a marker of vascular health, but they might contribute to vascular health. If this is the case, therapies designed to maintain persistently high levels of EPCs are strongly warranted in the next future.

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## Appendix

Search strategy: we performed a comprehensive literature search by using electronic bibliographic databases (MEDLINE, EMBASE, The Cochrane Library, and DARE) and combinations of the following keywords: endothelial progenitor cell, EPC, EPC mobilization, progenitor cell mobilization, cytokine, progenitor cells, myocardial ischaemia, myocardial infarction, characterization of EPC, CD34, KDR, ageing, oestrogens, risk factors, atherosclerosis, exercise, endothelial dysfunction, statins, ACE-inhibitors, AT II inhibitors, and nitrates. Articles were selected manually and bibliographies of all selected articles and review articles were reviewed for other relevant articles. Where necessary, study authors were contacted to obtain further data.

## References

- Cohnheim J. Über Entzündung und Eiterung [About inflammation and sepsis]. *Pathol Anat Physiol Klin Med* 1867;**40**:1–79.
- Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzensbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;**275**:964–967.
- Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;**279**:1528–1530.
- Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci USA* 1999;**96**:10711–10716.
- Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 2000;**6**:1229–1234.
- Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001;**105**:369–377.
- Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, Tschudy KS, Tilly JC, Cortes ML, Forkert R, Spitzer T, Iacomini J, Scadden DT, Tilly JL. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. *Cell* 2005;**122**:303–315.
- Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. *Circ Res* 2002;**91**:1092–1102.
- Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 2003;**9**:702–712.
- Li B, Sharpe EE, Maupin AB, Teleron AA, Pyle AL, Carmeliet P, Young PP. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. *FASEB J* 2006;**20**:1495–1497.
- He T, Smith LA, Harrington S, Nath KA, Caplice NM, Katusic ZS. Transplantation of circulating endothelial progenitor cells restores endothelial function of denuded rabbit carotid arteries. *Stroke* 2004;**35**:2378–2384.
- Stellos K, Gawaz M. Platelet interaction with progenitor cells: potential implications for regenerative medicine. *Thromb Haemost* 2007;**98**:922–929.
- Boos CJ, Lip GY, Blann AD. Circulating endothelial cells in cardiovascular disease. *J Am Coll Cardiol* 2006;**48**:1538–1547.
- Purhonen S, Palm J, Rossi D, Kaskenpää N, Rajantie I, Ylä-Herttua S, Alitalo K, Weissman IL, Salven P. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. *Proc Natl Acad Sci USA* 2008;**105**:6620–6625.
- Rodriguez-Menocal L, St-Pierre M, Wei Y, Khan S, Mateu D, Calfa M, Rahnama-Azar AA, Striker G, Pham SM, Vazquez-Padron RI. The origin of post-injury neointimal cells in the rat balloon injury model. *Cardiovasc Res* 2009;**81**:46–53.
- Timmermans F, Plum J, Yöder MC, Ingram DA, Vandekerckhove B, Case J. Endothelial progenitor cells: identity defined? *J Cell Mol Med* 2008 [Epub ahead of print]. doi:10.1111/j.1582-4934.2008.00598.x US:http://dx.doi.org/10.1111/j.1582-4934.2008.00598.x.
- Hristov M, Weber C. Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance. *J Cell Mol Med* 2004;**8**:508–515.
- Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Moore MA, Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34+ cells identifies a population of functional endothelial precursors. *Blood* 2000;**95**:952–958.
- Hill JM, Zalos G, Halcox JP, Schenke WH, Wacławski MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;**348**:593–600.
- Gulati R, Jevremovic D, Peterson TE, Chatterjee S, Shah V, Vile RG, Simari RD. Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. *Circ Res* 2003;**93**:1023–1025.
- Rehman J, Li J, Orschell CM, March KL. Peripheral blood 'endothelial progenitor cells' are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003;**107**:1164–1169.
- Hur J, Yoon CH, Kim HS et al. Characterization of two types of endothelial progenitor cells and their different contributions to neovascularogenesis. *Arterioscler Thromb Vasc Biol* 2004;**24**:288–293.
- Sieveking DP, Buckle A, Celermajor DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. *J Am Coll Cardiol* 2008;**51**:660–668.
- Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 2000;**105**:71–77.
- De Meyer SF, Vanhoorelbeke K, Chuah MK et al. Phenotypic correction of von Willebrand disease type 3 blood-derived endothelial cells with lentiviral vectors expressing von Willebrand factor. *Blood* 2006;**107**:4728–4736.
- Prater DN, Case J, Ingram DA, Yoder MC. Working hypothesis to redefine endothelial progenitor cells. *Leukemia* 2007;**21**:1141–1149.
- Yin AH, Miraglia S, Zanjan ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997;**90**:5002–5012.
- Leor J, Marber M. Endothelial progenitors: a new Tower of Babel? *J Am Coll Cardiol* 2006;**48**:1588–1590.
- Thum T, Fleissner F, Klink I, Tsikas D, Jakob M, Bauersachs J, Stichtenoth DO. Growth hormone treatment improves markers of systemic nitric oxide bioavailability via insulin-like growth factor-I. *J Clin Endocrinol Metab* 2007;**92**:4172–4179.
- Thum T, Tsikas D, Stein S, Schultheiss M, Eigenthaler M, Anker SD, Poole-Wilson PA, Ertl G, Bauersachs J. Suppression of endothelial progenitor cells in human coronary artery disease by the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine. *J Am Coll Cardiol* 2005;**46**:1693–1701.
- Rehman J, Li J, Parvathaneni L, Karlsson G, Panchal VR, Temm CJ, Mahenthiran J, March KL. Exercise acutely increases circulating endothelial progenitor cells and monocyte/macrophage-derived angiogenic cells. *J Am Coll Cardiol* 2004;**43**:2314–2318.
- Laufs U, Werner N, Link A, Endres M, Wassmann S, Jürgens K, Mische E, Böhm M, Nickenig G. Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. *Circulation* 2004;**109**:220–226.
- Laufs U, Urhausen A, Werner N, Scharhag J, Heitz A, Kissner G, Böhm M, Kindermann W, Nickenig G. Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy subjects. *Eur J Cardiovasc Prev Rehabil* 2005;**12**:407–414.
- Steiner S, Niessner A, Ziegler S, Richter B, Seidinger D, Pleiner J, Penka M, Wolzt M, Huber K, Wojta J, Minar E, Kopp CW. Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease. *Atherosclerosis* 2005;**181**:305–310.
- Strehlow K, Werner N, Berweiler J, Link A, Dirnagl U, Priller J, Laufs K, Ghaeni L, Milosevic M, Böhm M, Nickenig G. Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. *Circulation* 2003;**107**:3059–3065.
- Scheubel RJ, Zorn H, Silber RE, Kuss O, Morawietz H, Holtz J, Simm A. Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting. *J Am Coll Cardiol* 2003;**42**:2073–2080.
- Heiss C, Keymel S, Niesler U, Ziemann J, Kelm M, Kalka C. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 2005;**45**:1441–1448.
- Thum T, Hoeber S, Froese S, Klink I, Stichtenoth DO, Galuppo P, Jakob M, Tsikas D, Anker SD, Poole-Wilson PA, Bortak J, Ertl G, Bauersachs J. Age-dependent impairment of endothelial progenitor cells is corrected by growth-hormone-mediated increase of insulin-like growth-factor-1. *Circ Res* 2007;**100**:434–443.
- Henrich D, Hahn P, Wahl M, Wilhelm K, Dernbach E, Dimmeler S, Marzi I. Serum derived from multiple trauma patients promotes the differentiation of endothelial progenitor cells in vitro: possible role of transforming growth factor-beta1 and vascular endothelial growth factor165. *Shock* 2004;**21**:13–16.
- Dimmeler S, Vasa-Nicotera M. Aging of progenitor cells: limitation for regenerative capacity? *J Am Coll Cardiol* 2003;**42**:2081–2082.
- Dong C, Crawford LE, Goldschmidt-Clermont PJ. Endothelial progenitor cell obsolescence and atherosclerotic inflammation. *J Am Coll Cardiol* 2005;**45**:1458–1460.



42. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;**89**:E1–E7.
43. Michaud SE, Dussault S, Haddad P, Groleau J, Rivard A. Circulating endothelial progenitor cells from healthy smokers exhibit impaired functional activities. *Atherosclerosis* 2006;**187**:423–432.
44. Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, Iino S, Iden Y, Murohara T. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 2004;**24**:1442–1447.
45. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Böhm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;**353**:999–1007.
46. Imanishi T, Moriwaki C, Hano T, Nishio I. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J Hypertens* 2005;**23**:1831–1837.
47. Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH, Zhu JH. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. *Clin Sci (Lond)* 2004;**107**:273–280.
48. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, Menegolo M, de Kreutzenberg SV, Tiengo A, Agostini C, Avogaro A. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. *J Am Coll Cardiol* 2005;**45**:1449–1457.
49. Sorrentino SA, Bahlmann FH, Besler C, Müller M, Schulz S, Kirchhoff N, Doerries C, Horváth T, Limbourg A, Limbourg F, Fliser D, Haller H, Drexler H, Landmesser U. Oxidant stress impairs in vivo reendothelialization capacity of endothelial progenitor cells from patients with type 2 diabetes mellitus: restoration by the peroxisome proliferator-activated receptor- $\gamma$  agonist rosiglitazone. *Circulation* 2007;**116**:163–173.
50. Thum T, Fraccarollo D, Schulteiss M, Froese S, Galuppo P, Widder JD, Tsikas D, Ertl G, Bauersachs J. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes* 2007;**56**:666–674.
51. Verma S, Kuliszewski MA, Li SH, Szmítka PE, Zucco L, Wang CH, Badiwala MV, Mickle DA, Weisel RD, Fedak PW, Stewart DJ, Kutryk MJ. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. *Circulation* 2004;**109**:2058–2067.
52. Fujii H, Li SH, Szmítka PE, Fedak PW, Verma S. C-reactive protein alters antioxidant defenses and promotes apoptosis in endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2006;**26**:2476–2482.
53. Valgimigli M, Rigolin GM, Fucili A, Porta MD, Soukhomovskaia O, Malagutti P, Bugli AM, Bragotti LZ, Francolini G, Mauro E, Castoldi G, Ferrari R. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. *Circulation* 2004;**110**:1209–1212.
54. Deb A, Wang S, Skelding KA, Miller D, Simper D, Caplice NM. Bone marrow-derived cardiomyocytes are present in adult human heart: A study of gender-mismatched bone marrow transplantation patients. *Circulation* 2003;**107**:1247–1249.
55. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. *N Engl J Med* 2002;**346**:5–15.
56. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 2001;**103**:2776–2779.
57. Leone AM, Rutella S, Bonanno G, Abbate A, Rebuzzi AG, Giovannini S, Lombardi M, Galiuto L, Liuzzo G, Andreotti F, Lanza GA, Contemi AM, Leone G, Crea F. Mobilization of bone marrow-derived stem cells after myocardial infarction and left ventricular function. *Eur Heart J* 2005;**26**:1196–1204.
58. Massa M, Rosti V, Ferrario M, Campanelli R, Ramajoli I, Rosso R, De Ferrari GM, Ferlini M, Goffredo L, Bertolotti A, Klersy C, Pecci A, Moratti R, Tavazzi L. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood* 2005;**105**:199–206.
59. Müller-Ehmsen J, Scheid C, Grundmann F, Hirsch I, Turan G, Tossios P, Mehlhorn U, Schwinger RH. The mobilization of CD34 positive mononuclear cells after myocardial infarction is abolished by revascularization of the culprit vessel. *Int J Cardiol* 2005;**103**:7–11.
60. Gardini E, Merlini F, Malagutti P, Pratola C, Notarstefano P, Percoco G, Francolini G, Valgimigli M, Ferrari R. Modelli clinici di mobilizzazione delle cellule endoteliali progenitrici. *Ital Heart J* 2005;**6**(Suppl. 8):C149.
61. George J, Goldstein E, Abashidze S, Deutsch V, Shmilovich H, Finkelstein A, Herz I, Miller H, Keren G. Circulating endothelial progenitor cells in patients with unstable angina: association with systemic inflammation. *Eur Heart J* 2004;**25**:1003–1008.
62. Adams V, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, Tarnok A, Gielen S, Emmrich F, Schuler G, Hambrecht R. Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. *Arterioscler Thromb Vasc Biol* 2004;**24**:684–690.
63. Gasparidone A, De Fabritiis P, Scaffa R, Nardi P, Palombi F, Versaci F, Chiariello L. Stem cell mobilization after coronary artery bypass grafting. *Ital Heart J Suppl* 2004;**5**(Suppl. 1):23–28.
64. Shmilovich H, Deutsch V, Roth A, Miller H, Keren G, George J. Circulating endothelial progenitor cells in patients with cardiac syndrome X. *Heart* 2007;**93**:1071–1076.
65. Huang PH, Chen YH, Chen YL, Wu TC, Chen JW, Lin SJ. Vascular endothelial function and circulating endothelial progenitor cells in patients with cardiac syndrome X. *Heart* 2007;**93**:1064–1070.
66. Li M, Nishimura H, Iwakura A, Wecker A, Eaton E, Asahara T, Losordo DW. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via 'imported' nitric oxide synthase activity. *Circulation* 2005;**111**:1114–1120.
67. Kunz GA, Liang G, Cuculi F, Gregg D, Vata KC, Shaw LK, Goldschmidt-Clermont PJ, Dong C, Taylor DA, Peterson ED. Circulating endothelial progenitor cells predict coronary artery disease severity. *Am Heart J* 2006;**152**:190–195.
68. Güven H, Shepherd RM, Bach RG, Capoccia BJ, Link DC. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J Am Coll Cardiol* 2006;**48**:1579–1587.
69. Michowitz Y, Goldstein E, Wexler D, Sheps D, Keren G, George J. Circulating endothelial progenitor cells and clinical outcome in patients with congestive heart failure. *Heart* 2007;**93**:1046–1050.
70. Kissel CK, Lehmann R, Assmus B, Aicher A, Honold J, Fischer-Rasokat U, Heeschen C, Spyridopoulos I, Dimmeler S, Zeiher AM. Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. *J Am Coll Cardiol* 2007;**49**:2341–2349.
71. Leone AM, Rutella S, Bonanno G, Contemi AM, de Ritis DG, Giannico MB, Rebuzzi AG, Leone G, Crea F. Endogenous G-CSF and CD34+ cell mobilization after acute myocardial infarction. *Int J Cardiol* 2006;**111**:202–208.
72. George J, Goldstein E, Abashidze A, Wexler D, Hamed S, Shmilovich H, Deutsch V, Miller H, Keren G, Roth A. Erythropoietin promotes endothelial progenitor cell proliferative and adhesive properties in a PI 3-kinase-dependent manner. *Cardiovasc Res* 2005;**68**:299–306.
73. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, Dimmeler S. Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. *Blood* 2003;**102**:1340–1346.
74. Bahlmann FH, De Groot K, Spandau JM, Landry AL, Hertel B, Duckert T, Boehm SM, Menne J, Haller H, Fliser D. Erythropoietin regulates endothelial progenitor cells. *Blood* 2004;**103**:921–926.
75. Andreotti F, Coluzzi G, Cecchetti S, Lavorgna A, Marzo F, Crea F, Rumi C. Reduced CD34+, renal anemia, and adverse outcomes. *Am Heart J* 2006;**152**:e21.
76. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischemic cardiomyopathy. *Lancet* 2003;**362**:697–703.
77. De Falco E, Porcelli D, Torella AR, Straino S, Iachinoto MG, Orlandi A, Truffa S, Biglioli P, Napolitano M, Capogrossi MC, Pesce M. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. *Blood* 2004;**104**:3472–3482.
78. Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, Bosch-Marce M, Masuda H, Losordo DW, Isner JM, Asahara T. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 2003;**107**:1322–1328.
79. Rutella S, Pierelli L, Bonanno G, Scambia G, Leone G, Rumi C. Homogeneous expression of CXCR4 chemokine receptor 4 (CXCR4) on G-CSF-mobilized peripheral blood CD34+ cells. *Blood* 2000;**95**:4015–4016.
80. Min TQ, Zhu CJ, Xiang WX, Hui ZJ, Peng SY. Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther* 2004;**18**:203–209.
81. Wang CH, Verma S, Hsieh IC, Chen YJ, Kuo LT, Yang NI, Wang SY, Wu MY, Hsu CM, Cheng CW, Cherng WJ. Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. *J Mol Cell Cardiol* 2006;**41**:34–43.
82. Bahlmann FH, de Groot K, Mueller O, Hertel B, Haller H, Fliser D. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* 2005;**45**:526–529.

83. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, Dimmeler S. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 2001;**103**:2885–2890.
84. Assmus B, Urbich C, Aicher A, Hofmann WK, Haendeler J, Rössig L, Spyridopoulos I, Zeiher AM, Dimmeler S. HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells via regulation of cell cycle regulatory genes. *Circ Res* 2003;**92**:1049–1055.
85. Oeseburg H, Westenbrink BD, de Boer RA, van Gilst WH, van Veldhuisen DJ, van der Harst P. Can critically short telomeres cause functional exhaustion of progenitor cells in postinfarction heart failure? *J Am Coll Cardiol* 2007;**50**:1911–1912.
86. Leone AM, Rutella S, Giannico MB, Perfetti M, Zacccone V, Brugaletta S, Garramone B, Niccoli G, Porto I, Liuzzo G, Biasucci LM, Bellesi S, Galiuto L, Leone G, Rebuzzi AG, Crea F. Effect of intensive vs standard statin therapy on endothelial progenitor cells and left ventricular function in patients with acute myocardial infarction: statins for regeneration after acute myocardial infarction and PCI (STRAP) trial. *Int J Cardiol* 2008;**130**:457–462.
87. Hristov M, Fach C, Becker C, Heussen N, Liehn EA, Blindt R, Hanrath P, Weber C. Reduced numbers of circulating endothelial progenitor cells in patients with coronary artery disease associated with long-term statin treatment. *Atherosclerosis* 2007;**192**:413–420.
88. Schwartz GG, Olsson AG, Ezekowitz MD, Ganz P, Oliver MF, Waters D, Zeiher A, Chaitman BR, Leslie S, Stern T. Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study Investigators. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. *JAMA* 2001;**285**:1711–1718.
89. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM; Pravastatin or Atorvastatin Evaluation Infection Therapy-Thrombolysis in Myocardial Infarction 22 Investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* 2004;**350**:1495–1504. Erratum in: *N Engl J Med* 2006;**354**:778.
90. Thum T, Fraccarollo D, Galuppo P, Tsikas D, Frantz S, Ertl G, Bauersachs J. Bone marrow molecular alterations after myocardial infarction: Impact on endothelial progenitor cells. *Cardiovasc Res* 2006;**70**:50–60.
91. Humpert PM, Neuwirth R, Battista MJ, Voronko O, von Eynatten M, Konrade I, Rudofsky G Jr, Wendt T, Hamann A, Morcos M, Nawroth PP, Bierhaus A. SDF-1 genotype influences insulin-dependent mobilization of adult progenitor cells in type 2 diabetes. *Diabetes Care* 2005;**28**:934–936.
92. Humpert PM, Djuric Z, Zeuge U, Oikonomou D, Seregin Y, Laine K, Eckstein V, Nawroth PP, Bierhaus A. Insulin stimulates the clonogenic potential of angiogenic endothelial progenitor cells by IGF-1 receptor-dependent signaling. *Mol Med* 2008;**14**:301–308.
93. Pistrosch F, Herbrig K, Oelschlaegel U, Richter S, Passauer J, Fischer S, Gross P. PPARgamma-agonist rosiglitazone increases number and migratory activity of cultured endothelial progenitor cells. *Atherosclerosis* 2005;**183**:163–167.
94. Gensch C, Clever YP, Werner C, Hanhoun M, Böhm M, Laufs U. The PPAR-gamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells. *Atherosclerosis* 2007;**192**:67–74.
95. Redondo S, Hristov M, Gümbel D, Tejerina T, Weber C. Biphasic effect of pioglitazone on isolated human endothelial progenitor cells: involvement of peroxisome proliferator-activated receptor-gamma and transforming growth factor-beta1. *Thromb Haemost* 2007;**97**:979–987.
96. DiFabio JM, Thomas GR, Zucco L, Kuliszewski MA, Bennett BM, Kutryk MJ, Parker JD. Nitroglycerin attenuates human endothelial progenitor cell differentiation, function, and survival. *J Pharmacol Exp Ther* 2006;**318**:117–123.
97. Thum T, Fraccarollo D, Thum S, Schultheiss M, Daiber A, Wenzel P, Munzel T, Ertl G, Bauersachs J. Differential effects of organic nitrates on endothelial progenitor cells are determined by oxidative stress. *Arterioscler Thromb Vasc Biol* 2007;**27**:748–754.
98. Schmidt-Lucke C, Rössig L, Fichtlscherer S, Vasa M, Britten M, Kämper U, Dimmeler S, Zeiher AM. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 2005;**111**:2981–2987.
99. Wojakowski W, Tendera M, Michałowska A, Majka M, Kucia M, Maślankiewicz K, Wyderka R, Ochala A, Ratajczak MZ. Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation* 2004;**110**:3213–3220.
100. Wojakowski W, Tendera M, Zebzda A, Michałowska A, Majka M, Kucia M, Maślankiewicz K, Wyderka R, Król M, Ochala A, Kozakiewicz K, Ratajczak MZ. Mobilization of CD34(+), CD117(+), CXCR4(+), c-met(+) stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction. *Eur Heart J* 2006;**27**:283–289.
101. Valgimigli M, Biondi-Zoccai GGL, Malagutti P, Leone AM, Abbate A. Autologous bone marrow stem cell mobilization induced by granulocyte colony-stimulating factor after myocardial infarction. *Eur Heart J Suppl* 2008;**10**:K27–K34.
102. Zohnhöfer D, Dibra A, Koppa T, de Waha A, Ripa RS, Kastrup J, Valgimigli M, Schömig A, Kastrati A. Stem cell mobilization by granulocyte colony-stimulating factor for myocardial recovery after acute myocardial infarction: a meta-analysis. *J Am Coll Cardiol* 2008;**51**:1429–1437.
103. Abdel-Latif A, Bolli R, Zuba-Surma EK, Tleyjeh IM, Hornung CA, Dawn B. Granulocyte colony-stimulating factor therapy for cardiac repair after acute myocardial infarction: a systematic review and meta-analysis of randomized controlled trials. *Am Heart J* 2008;**156**:216–226.
104. Xiao Q, Kiechl S, Patel S, Oberhollenzer F, Weger S, Mayr A, Metzler B, Reindl M, Hu Y, Willeit J, Xu Q. Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis—results from a large population-based study. *PLoS ONE* 2007;**2**:e975.