

# REVIEW ARTICLE

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## Bone marrow–derived cells and arterial disease

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This article reviews the association between bone and artery disease, with particular relevance to progenitor cells. The review was based on insight gained by analysis of previous publications and on-going work by the authors. A large number of studies have demonstrated a correlation between bone pathology, particularly osteoporosis, and atherosclerosis. In this review we highlight the particular aspect of bone marrow progenitor cells in the bone-artery link. Progenitor cells, primarily those believed to give rise to endothelial cells, have been inversely correlated with atherosclerosis severity and risk factors. Therapeutic approaches aimed at manipulating progenitor cells in revascularization and vascular repair have demonstrated some promising results. Subtypes of progenitor cells have also been linked with vascular pathology, however, and further studies are required to assess relative beneficial and pathologic effects of bone marrow–derived progenitors. Further understanding of the link between bone and artery pathophysiology is likely to be of significant value in developing new therapies for vascular disease. (*J Vasc Surg* 2007;46:590-600.)

Bone marrow–derived monocyte-macrophages have been demonstrated to be central in the pathogenesis of a number of arterial diseases, including atherosclerosis and intimal hyperplasia. Interest in the link between bone and artery disease, however, has become much more marked with the demonstration that bone marrow stem cells can give rise to endothelial and vascular smooth muscle cells (VSMCs). The presence of cells and matrix within the artery wall in vascular disease, which are often associated with bone remodelling, suggests an intimate, yet complex interaction between these two tissues. In this review we discuss the present evidence linking bone and artery and in particular highlight the importance of bone marrow derived progenitor cells.

### ASSOCIATION BETWEEN BONE PATHOLOGY AND ARTERIAL DISEASE

Cardiovascular disease has been associated with a number of bone pathologies, including Paget disease, osteoporosis, and renal osteodystrophy.<sup>1-3</sup> The most consistent association has been demonstrated between osteoporosis and atherosclerosis, particularly arterial mineralization.<sup>3-10</sup> A number of studies have demonstrated an association

between the severity of bone mineral density loss and vascular calcification or other measures of atherosclerosis.<sup>4,9</sup> Low bone mineral density has been associated with previous myocardial infarction (MI), carotid atherosclerosis, arterial stiffness, and subsequent cardiovascular events.<sup>5,7,8,10</sup> Jorgensen et al<sup>6</sup> found in a prospective study of 2733 women that subjects with echogenic carotid plaques were more at risk of subsequent fractures during a 6-year follow-up. Bagger et al<sup>4</sup> similarly demonstrated an independent association between aortic calcification and subsequent bone loss and fracture risk.<sup>4</sup> The incidence of femur fractures has been correlated with coronary calcification.<sup>3</sup>

Mechanisms underlying the association between bone and artery pathology (Fig 1) may include:

- (a) *Common risk factors:* Age, genetic associations, dyslipidemia, oxidative stress, inflammation, hyperhomocystinemia, hypertension, diabetes, and smoking have been associated with bone mineral density loss in some but not all studies.<sup>11-14</sup> These factors are well-recognized risk factors for atherosclerosis.
- (b) *Related but contrasting biology:* Significant advances in the understanding of bone biology have identified a number of processes in bone turnover related to atherosclerosis. Examples of proteins important in regulating bone homeostasis and remodelling that have been implicated in atherosclerosis include matrix Gla protein, osteoprotegerin, receptor activator of necrosis factor- $\kappa$ B ligand, osteopontin, and bone morphogenic proteins, among many others.<sup>15,16</sup> Possibly as a result of the different environments of bone and artery, these molecules appear to play different roles in the two sites. For example, osteoprotegerin protects bone against

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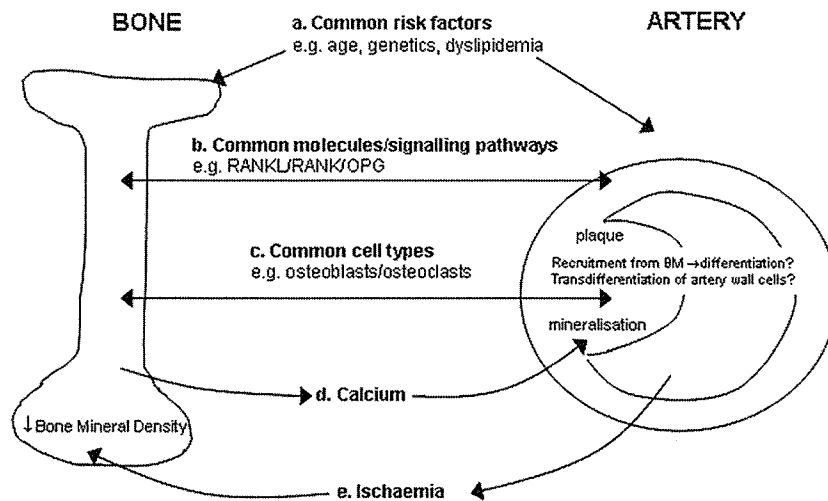
Competition of interest: none.

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**Fig 1.** Bone and artery pathologies share common risk factors and mechanisms. Bone and arterial pathologies share (a) common risk factors including age, genetic associations, dyslipidemia, inflammation, hypertension, diabetes and smoking; (b) common signalling pathways including the receptor activator of nuclear factor  $\kappa$ B ligand (*RANKL*)/receptor activator of nuclear factor  $\kappa$ B (*RANK*)/osteoprotegerin (*OPG*) system, which is associated with both bone remodelling and arterial pathology; and (c) similar cells, including osteoblasts and osteoclasts involved in bone remodelling and reported in calcified vascular lesions. The release of (d) calcium during the bone demineralization process of some bone pathologies, including osteoporosis, may have a role in passive mineralization of the artery wall. Occlusion of blood vessels mediated by (e) atherosclerotic plaque may result in ischemia of bone and decreased bone mineral density.

breakdown but appears to inhibit arterial calcification. In addition, oxidative stress factors such as oxidized lipoproteins have been shown to modulate vascular and bone cells differently. Oxidative stress enhances osteoblastic differentiation of calcifying vascular cells but inhibits that of bone cell. These opposing effects may contribute to the association of bone destruction with arterial mineralization.<sup>17</sup>

(c) *Association of cellular elements:* The association of bone-related factors with atherosclerosis and the presence of mineralized bone deposits within atherosclerotic plaques suggests the potential of cells present within the artery wall to promote osteogenesis.<sup>18</sup> Cells important in bone turnover, including osteoblasts and osteoclast-like cells, have counterparts in the artery wall.<sup>18</sup> In addition, a number of different cell types found within the artery wall, including VSMCs, have osteogenic potential under experimental conditions in vitro.<sup>19</sup> Whether these bone-remodelling cells have differentiated from existing cells in the artery wall, under the influence of injury-induced local changes and factors, or are the progeny of bone marrow stem cells seeding sites of vascular injury is unclear. However, the finding that bone marrow-derived cells are involved in both vascular repair and pathology suggests a complex relationship between these bone marrow progenitor cells and the artery wall.<sup>20</sup> These cellular links are the focus of this review.

(d) *Passive mineral based link:* The systemic release of bone products such as calcium could lead to their passive

deposition in arteries. The focal nature of the mineralization, the incorporation of elements of bone, including lamellar bone, and the associated matrix proteins make this mechanism unlikely to be of primary importance, despite some evidence from animal models.<sup>18,21</sup>

(e) *Effects of atherosclerosis on bone blood supply:* Loss of bone mineral density due to atherosclerotic occlusion of bone blood supply has been suggested as a further link between vascular and bone disease.<sup>4</sup>

## PROGENITOR CELLS

### Identification of progenitor cells in humans.

Progenitor cells are naive cells capable of infinite renewal before differentiation into more mature populations. A number of different cell populations isolated from bone marrow and peripheral blood of humans and animal models have been demonstrated to differentiate into endothelial cells, VSMCs, blood cells, and osteocytes in vitro.<sup>22</sup> This has led to the concept of circulating progenitor cells for endothelium (EPCs), VSMCs (SPCs), and bone or ectopic calcification. These cell types remain controversial, and there is debate about how they should be identified, what roles they play in physiology and disease, and whether they can be manipulated for therapeutic purposes.<sup>20,23</sup> Identification of progenitor cells is confounded by their remarkable lineage plasticity; that is, they can differentiate into a variety of progeny once stimulated appropriately. Commonly used methods of identification of progenitor cells include flow cytometry, cell culture, or a combination of the two (Table I).<sup>20,24-28</sup>

**Table I.** Human progenitor cells

Progenitor	Frequency in peripheral blood	Surface markers present	Surface markers absent	Behavior in culture
HSC <sup>25</sup>	1/10-100,000 blood cells	CD34*	Lineage	Nonadherent, can differentiate into multiple lineages: red blood cells, monocytes, leukocytes
MSC <sup>25</sup>	Less than HSC	CD105, CD73	CD45	Adherent, can differentiate into multiple cell types: osteocytes, chondrocytes, adipocytes
EPC <sup>20</sup>	Less than HSC	CD34, CD133, VEGFR-2*		Differentiate into endothelial cells, form tubes on matrigel, highly proliferative, can be genetically modified
CEC <sup>24</sup>	1-10 cells/mL	CD146, Ulex europaeus lectin 1	CD34, CD133	Maybe apoptotic or necrotic
SPC <sup>27</sup>	Unknown	$\alpha$ SMA, myosin heavy chain, calponin, CD34, Flk-1, VEGFR-2, $\alpha_5\beta_1$	Tie-2 receptor, CD31	Hill & valley appearance after 2 weeks PDGFBB
OPC <sup>26</sup>	1%-2% of PBMNCs	OC, BAP		Form mineralization nodules in vitro & in vivo

HSC, Hematopoietic stem cell; MSC, mesenchymal stem cell; EPC, endothelial progenitor cell; SPC, vascular smooth muscle cell progenitor cell; CEC, circulating endothelial cell; BM, bone marrow; CSFs, colony stimulating factors; VEGF(R2), vascular endothelial growth factor (receptor-2) or KDR in humans, flk-1 in mice;  $\alpha$ SMA, smooth muscle cell-specific  $\alpha$  actin; MMP-9, matrix metalloproteinase-9; PDGF-BB, platelet-derived growth factor-BB; PBMNCs, peripheral blood mononuclear cells; OC, osteocalcin; BAP, bone specific alkaline phosphatase; OPC, osteogenic progenitor cell.

\*CD34<sup>+</sup> HSC/EPCs also described, CD34<sup>+</sup> EPC suggested to have more re-endothelialization potential by some investigators.<sup>20</sup> Lineage markers include mature leukocyte markers such as CD45 and CD14.

Cells isolated from tissues, including skeletal muscle, heart, and adventitia have also been shown to differentiate into endothelial and VSMCs in vitro.<sup>29,30</sup> In the embryo, VSMCs can arise as a result of transdifferentiation of endothelium or directly from mesoderm or neuroectodermal (aortic arch).<sup>31,32</sup> In the adult, it is also likely that vascular cells (endothelial cells and VSMCs) arise from a variety of sources, as emphasized by the ability of both donor and recipient cells to generate outgrowth endothelial cells in peripheral blood mononuclear cells from human bone marrow transplant patients.<sup>33</sup>

At present, the most convincing data support the existence of EPCs: large numbers of review articles and high-impact publications have appeared since their original discovery in 1997.<sup>20,28,34</sup> Even for this cell type, however, there is controversy about identification.<sup>35</sup> For example, circulating endothelial cells (which may be sloughed off an inflamed endothelium during apoptosis or necrosis) or microparticles of these have been identified by some investigators and their presence interpreted as a measure of arterial disease severity.<sup>24</sup>

**Control of progenitor cells.** The maintenance and mobilization of stem cells in the bone marrow is determined by the local microenvironment, the "stem cell niche," which consists of bone marrow stromal cells such as osteoblasts, endothelial cells, and fibroblasts.<sup>36</sup> Their release from this niche and mobilization into the circulation requires a complex sequence of events and cell types. Adhesive interactions between stem cells and stromal cells are responsible for stem cell homing to the bone marrow and

include vascular cell adhesion molecule-1 and the CXC chemokine receptor 4/stromal cell-derived factor-1 chemotactic axis.

Mobilizing factors reverse the homing process by increasing the motility of the stem cells and facilitating their release from the stromal cells, which enables stem cells to leave the bone marrow by transendothelial migration.<sup>37</sup> Difficulties in precisely discriminating the various progenitor cell types within the mononuclear fraction of bone marrow and peripheral blood, such as EPC, SPCs, hemopoietic stem cells (HSCs), and their multipotent ancestors, has hindered definitive descriptions of the specific mechanisms that each use to exit the bone marrow. Peripheral blood EPCs and SPCs differ in their expression of surface integrins and attachment to extracellular matrix proteins. Whether these differences also occur within the bone marrow and their release is modulated differently is unknown.<sup>38</sup> Arrival of progenitor cells to the sites of vascular disease can be considered to involve their mobilization from the bone marrow or other resident sites, recruitment to the artery, and differentiation within the vessel. Control of these different processes will be discussed in the following paragraphs (Fig 2).

**Mobilization.** Mature immune cells, including myeloid and lymphoid cells, are continually released from the bone marrow into the circulation during normal homeostasis, whereas very low numbers of primitive progenitor cells enter the circulation under these conditions. Vascular injury, tissue ischemia, exercise, and tumor growth have all been associated with increased mobilization of vascular

Table I. Continued

Origin	Mobilization	Destinations, roles
BM	CSFs, SDF-1	Many roles: oxygen carriage, inflammation
BM, possibly other tissues	Unclear	Repair versus disease, immunosuppression, HSC engrafting & hematopoiesis
BM putative hemangioblast, possible common HSC & EPC <sup>28</sup>	Tissue ischemia, MMP-9, G-CSF, SDF-1, VEGF	Endothelial repair, vasculogenesis
Damaged endothelium	Atherosclerosis, inflammation	Marker of disease state
BM	Unknown	Unknown: possibly atherosclerosis, intimal hyperplasia, vascular repair
BM	Unknown	Unknown

progenitor cells.<sup>22</sup> Progenitor cell mobilization from the bone marrow is mainly mediated by factors increasing the activity of proteinases, such as elastase, cathepsin G, and the matrix metalloproteinases (Table I). For example, granulocyte colony-stimulating factor (G-CSF) releases elastase and cathepsin from neutrophils, which cleave a number of proteins that keep progenitor cells resident within the bone marrow, including vascular cell adhesion molecule-1, stromal cell-derived factor-1, and CXC chemokine receptor 4.<sup>39,40</sup> This results in loss of the adhesive bond between bone marrow stromal cells and progenitor cells, thereby liberating these cells from their stem cell niche. In addition, stromal cell-derived factor-1 and vascular endothelial growth factor (VEGF) induce matrix metalloproteinase-9, which cleaves the membrane-bound kit ligand resulting in the release of soluble kit ligand. Soluble kit ligand confers signals to enhance the mobility of progenitor cells, promoting their movement into the circulation.<sup>41</sup>

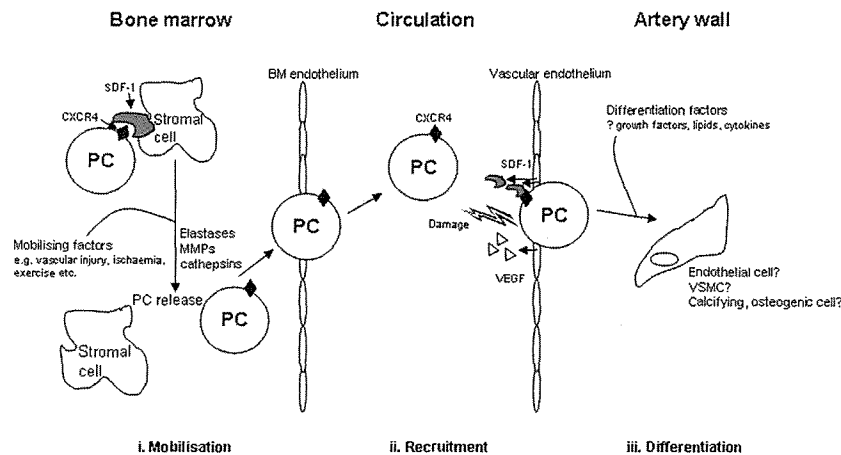
**Recruitment.** After mobilization, circulating vascular progenitor cells home to sites of injured ischemic tissue or growing malignant tumors, accelerating the tissue repair and revascularization process.<sup>42</sup> This multistep process of chemoattraction, adhesion, transmigration, and differentiation has not yet been fully elucidated, although it likely shares many characteristics with leukocyte infiltration. Important factors in the chemoattraction of EPCs include VEGF and stromal cell-derived factor-1. VEGF is upregulated after MI and attracts the VEGF receptor-positive EPCs to the site of ischemia.<sup>43</sup>

Similarly, endothelial cells respond to damage by release of stromal cell-derived factor-1, a process con-

trolled by hypoxia-inducible factor-1, which thus creates a chemotactic gradient for CXC chemokine receptor 4-positive vascular progenitor cells to injured and ischemic tissue.<sup>44</sup> Molecules believed to be important in the adhesion of progenitor cells to the target tissue include selectins, integrins, CD31, and monocyte chemoattractant protein-1.<sup>22</sup>

The control of recruitment of SPCs from the bone marrow is less clear and controversial, particularly as the precise origin of VSMCs has not been clarified. Some authors have suggested that VSMCs in the neointima of vascular graft lesions are derived from tissue-resident progenitor cells, whereas others have demonstrated a significant proportion of VSMCs in plaques were derived from donor bone marrow in transplant experiments, suggesting the recruitment of bone marrow-derived SPCs.<sup>45-47</sup>

**Differentiation.** VEGF has been implicated in endothelial differentiation.<sup>28</sup> The genetic pathways underlying progenitor cell differentiation are being explored in mice models. For example, the protein kinase Pim-1 and the transcription factor Hex are required for EPC differentiation,<sup>48,49</sup> whereas transforming growth factors  $\beta 1$  and  $\beta 3$  and platelet-derived growth factor BB are implicated in SPC differentiation.<sup>22</sup> Debate continues about whether transdifferentiation occurs.<sup>50</sup> Similarly, there is controversy about whether cells can de-differentiate from a committed but not terminally differentiated lineage to a more primitive multipotent progenitor in the adult vessel wall and are capable of significant changes in their phenotypes in response to alterations in their local environment.<sup>51</sup>



**Fig 2.** Circulating progenitor cells (PC) as a link between the bone and artery. Progenitor cells are maintained in the bone marrow stem cell niche via their interactions with stromal cells such as osteoblasts, endothelial cells, and fibroblasts. CXC chemokine receptor 4 (*CXCR4*)/stromal cell derived factor-1 (*SDF-1*) interactions are one of the important receptor/ligand pairs that maintain progenitor cells in the bone marrow. (i) Mobilizing factors including vascular injury, inflammation, tissue ischemia, or mobilization treatments like granulocyte colony stimulating factor result in the production of proteinases from various cells, which alter the progenitor cell/stromal cell association. Progenitor cells leave the stem cell niche via transendothelial migration and enter the peripheral circulation. (ii) Vascular endothelial cells release chemoattractant molecules, including vascular endothelial growth factor (*VEGF*), and *SDF-1* in response to endothelial damage, which recruits inflammatory cells and potentially progenitor cells from the circulation. (iii) Recruited progenitor cells may traverse the endothelium via a diapedesis-like process and enter the underlying intima. Here, under the influence of unknown differentiation factors progenitor cells may differentiate into endothelial cells, VSMCs, or osteogenic cells capable of mineralization.

## ASSOCIATION BETWEEN PROGENITOR CELLS AND ARTERIAL DISEASE

### Endothelium progenitor cells and artery disease

**Animal data.** A study of bone marrow transplantation in an animal model of mice expressing green fluorescent protein reported that donor cells give rise to new endothelium after vascular injury.<sup>52</sup> Factors associated with EPC mobilization, including G-CSF and statins, have been related to increased speed of re-endothelialization and reduced intimal hyperplasia.<sup>53,54</sup> Bone marrow from younger nonatherosclerotic mice has been demonstrated to reduce atheroma development in older mice.<sup>55,56</sup> These studies support the ability of bone marrow-derived EPC to effect vascular repair, thereby reducing the development of intimal hyperplasia and atheroma.

**Human data.** Data from a large number of studies in human subjects suggests that EPC numbers are reduced in atherosclerosis in relation to its severity and risk factors.<sup>57-63</sup> For example, bone marrow-derived mononuclear cells have reduced ability to form EPC colony-forming units and migration when removed from patients with heart failure compared with healthy controls.<sup>59</sup> EPC number has been inversely correlated with carotid intima-media thickness, severity of coronary artery disease, lower limb ischemia, transplant atherosclerosis, and cerebrovascular disease, and positively associated with endothelial dependent relaxation.<sup>57,60,62,64-66</sup> Composite cardiovascular risk factor scores and individual predictors of atherosclerosis have

been extensively associated with reduced number and function of EPC; for example, hypertension, homocysteine, C-reactive protein, renal failure, hypercholesterolemia, angiotensin II, diabetes, and smoking.<sup>58,62,67-71</sup>

Although factors demonstrated to be protective against atherosclerosis have been correlated with increased numbers of EPCs, such as high-density lipoprotein, estrogen, statins, and angiotensin II inhibitors,<sup>54,69,72,73</sup> also of importance is that EPC numbers have been shown to predict subsequent cardiovascular events. Schmidt-Lucke et al,<sup>61</sup> in a study of 120 individuals followed up for a median of 10 months, demonstrated the number of EPCs to be independently negatively correlated with the risk of cardiovascular events.<sup>61</sup> In a study of 519 patients with coronary artery disease, Werner et al<sup>74</sup> demonstrated that cardiovascular events were associated with EPC tertiles. After adjustment for other risk factors, increased EPC number was associated with lower cardiovascular death at a hazard ratio of 0.31 (95% confidence interval, 0.16 to 0.63).<sup>74</sup>

Thus, it has been suggested that impaired EPC repair of recurrent endothelial injury is instrumental in atherosclerosis development and progression.<sup>20</sup> In contrast to the summarized findings just presented, a recent study reported that the number of circulating EPCs was greater in patients with abdominal aortic aneurysm compared with age-matched controls.<sup>75</sup> Whether the role of EPCs in occlusive atherosclerosis and aortic aneurysm is different is presently unclear. Further studies focusing on the tracking of EPCs will be

required to resolve the controversy regarding this cell population.

#### Smooth muscle progenitor cells and artery disease

**Animal models.** A number of potential sources of SPCs have been suggested, including bone marrow (HSC and mesenchymal stem cells [MSCs]), adventitia, skeletal, and cardiac muscle.<sup>76</sup> To track the destination of SPCs, most investigators have used a protocol involving the injection of labelled donor bone marrow into lethally irradiated animals, particularly mice. The presence of labelled donor cells within arterial disease sites is then inferred as being identical to the total contribution made by the bone marrow in a nonirradiated animal. Irradiation may not eradicate all recipient bone marrow, however, meaning that these studies could underestimate the bone marrow contribution to artery disease. For example, a number of studies have suggested that stromal cells, which could give rise to MSCs, remain after irradiation.<sup>77</sup>

A few studies have investigated the effect of injecting mononuclear cells in the absence of irradiation (Table II).<sup>56,78,79</sup> A number of studies have supported the role of bone marrow-derived SPCs in arterial pathologies, including transplant atherosclerosis, intimal hyperplasia, and atherosclerosis associated with hyperlipidaemia.<sup>45,47,80-83</sup> A recent study using high-resolution microscopy has questioned the findings from these earlier investigations. Bentzon et al<sup>84</sup> studied atheroma in apolipoprotein E deficient (ApoE<sup>-/-</sup>) mice that had their bone marrow reconstituted from sex-mismatched ApoE<sup>-/-</sup> green fluorescent protein-positive animals. Using confocal microscopy, the investigators reported that no green fluorescent protein or Y chromosome-positive VSMCs were present in approximately 10,000  $\alpha$ -actin positive cells examined. Sites assessed included spontaneous plaques and intimal hyperplasia induced by placement of transplanted segments of arteries in the presence of an external cuff. A number of other studies have also failed to find significant bone marrow contribution to artery disease, although it is difficult to know how the findings in these different models relate to human atherosclerosis.<sup>85,86</sup>

**Human data.** VSMCs have been grown from human peripheral blood mononuclear cells.<sup>27</sup> Caplice et al,<sup>87</sup> using fluorescence in situ hybridization to identify X and Y chromosomes in atheroma excised from patients who had previously received sex-mismatched bone marrow transplants, reported that 10% of VSMCs within atherosclerotic arteries were of donor origin.<sup>87</sup> Further studies are required to resolve the importance of SPCs in human atherosclerosis and intimal hyperplasia.

#### Osteoblastic progenitor cells and artery disease

More recently, progenitor cells positive for osteocalcin and bone-specific alkaline phosphatase have been identified as osteoblastic progenitor cells (OPCs) in the peripheral blood. These cells not only express bone-related proteins but are also capable of adherence, replication, and formation of mineralized nodules in vitro as well as forming bone

in an in vivo mice transplantation assay.<sup>26</sup> The origin of these cells is believed to be in the nonadherent bone marrow population, which contains a pool of cells with osteogenic potential as well as cells with both hematopoietic and mesenchymal (eg, osteoblastic) reconstituting ability.<sup>88</sup> OPCs represent 1% to 2% of the mononuclear cells in peripheral circulation, and their number increases during times of increased bone formation such as pubertal growth or after bone fracture.<sup>26</sup>

In addition to contributing to physiologic bone formation, the presence of osteoblastic cells in peripheral blood suggests their possible role in the pathogenesis of ectopic ossification. Vascular calcification, for example, seems to be an active, cell-mediated process involving osteoblast-like cells. Because the naive vasculature contains no osteoblasts, it has been suggested that deregulated mobilization, homing, and proliferation of circulating OPCs may play a role in the calcification of blood vessels and heart valves as a common complication of atherosclerosis.<sup>89</sup> Further studies are required to support or refute these theories, particularly as there is increasing interest in injecting fractions of bone marrow, which could thus be associated with vascular calcification.<sup>90</sup>

#### Other progenitor cells and artery disease

In addition to progenitor cells, HSC and MSC have been suggested to play a role in vascular physiology and pathology (Table I). HSC are multipotent stem cells that give rise to all cells of the blood lineage, whereas MSC are capable of differentiating into various other cell types, including osteocytes, chondrocytes, fibroblasts, myocytes, and adipocytes. In recent years, it has been proposed that these cells can circulate to the site of injury, where they contribute to myocardial repair and regeneration. Numerous groups have reproduced the observation of stem cell-induced angiogenesis, but the possibility of myogenesis has also been suggested.<sup>91</sup> In culture conditions and animal models, the differentiation of MSC to cardiomyocyte-like cells is well documented, but extrapolation to clinical settings faces a variety of obstacles and uncertainties. When injected into infarct tissue, MSCs may enhance regional wall motion and prevent remodelling of the remote, non-infarcted myocardium.<sup>92</sup> The secretion of angiogenic cytokines by MSC may play an important role in their angiogenic properties.<sup>93</sup> Organ-resident stem cells have also been identified such as cardiac or adventitial, but their role in physiology and pathology requires further investigation.

#### THERAPEUTIC MANIPULATION OF PROGENITOR CELLS

Given our present knowledge of the function and mechanisms controlling movement of progenitor cells, a number of therapeutic benefits of manipulating them have been suggested and investigated. Although most of the research has been aimed at EPCs, most studies have involved relatively crude isolates of cells. In theory, it would be possible to influence progenitor cells by altering mobilization, recruitment, and differentiation. Studies to date

**Table II.** Studies examining the effect of bone marrow injection without irradiation in mice models of atherosclerosis

Study	Donor species	Donor age	Cell type	No.	Recipient species	Recipient age
78	ApoE <sup>-/-</sup>	10 wks	BM	10 <sup>6</sup>	ApoE <sup>-/-</sup>	10 wks
78	ApoE <sup>-/-</sup>	10 wks	Splenic MNCs <sup>‡</sup>	10 <sup>6</sup>	ApoE <sup>-/-</sup>	10 wks
56	ApoE <sup>-/-</sup>	4 wks (6 mon)	BMU/A enriched	2 × 10 <sup>6</sup>	ApoE <sup>-/-</sup>	3 wks
79	C57BL/6 (GFP)	NS	BM-MNCs	10 <sup>6</sup>	ApoE <sup>-/-</sup> & ischemia	14 wks
79	ApoE <sup>-/-</sup>	NS	BM-MNCs	10 <sup>6</sup>	ApoE <sup>-/-</sup> & ischemia	14 wks

NS, Not stated; MNCs, mononuclear cells; BM, bone marrow cells unspecified; BMU, nonplastic adherent BM cells (hematopoietic enriched); BMA, plastic adherent BM cells (stromal/ mesenchymal); ApoE<sup>-/-</sup>, apolipoprotein E deficient mice; GFP, green fluorescent protein.

\*Atherosclerosis was measured by microscopic area at the aortic sinus in all studies.

<sup>†</sup>*P* < .01 compared with control group.

<sup>‡</sup>Cultured in endothelial media to convert to endothelial progenitor cells for 5 days.

have mainly concentrated on either trying to mobilize cells or injecting them.

### Revascularization

**Animal studies.** A number of investigators have studied the effect of isolation of fractions of mononuclear cell populations using surface markers (eg, Flk-1), expansion ex vivo under preferential growth conditions with or without genetic manipulation, and injection. For example, Kawamoto et al<sup>94</sup> demonstrated that human peripheral blood mononuclear cells, expanded ex vivo when injected 4 weeks after MI induction in a rat model, improved the histologic appearance and function of the heart. Similarly, CD34-positive (not CD34-negative) GCSF-mobilized human peripheral blood mononuclear cells injected intravenously in rats with ischemic myocardium led to new vessel formation plus improved myocardial function.<sup>95</sup> Kalka et al<sup>96</sup> reported that injecting human peripheral blood mononuclear cells into athymic mice in which the femoral artery had been excised led to limb salvage in 59% compared with 8% in controls.

Similar findings have been reported in athymic rats and mice receiving stromal cell derived factor-1 injections.<sup>97,98</sup> The mechanisms underlying these benefits are likely a combination of a low rate of incorporation of EPCs leading to local vasculogenesis, and release of cytokines and chemokines promoting angiogenesis.<sup>86,99</sup> The incorporation of EPC in mice is reported to be only around 1% of injected cells in basal conditions but can increase to >10% during endothelial injury.<sup>100</sup>

**Human studies.** A number of trials have investigated the effect of intracoronary and peripheral injection of mononuclear cells in the treatment of MI, heart failure, and lower limb ischemia.<sup>101,102</sup> In the biggest study 204 patients were randomized after acute MI between intracoronary injection of bone marrow-derived mononuclear cells or placebo medium.<sup>103</sup> The authors reported a reduction in the combined end point of death, recurrence of MI, or revascularization from 40% to 23% at 1 year (*P* = .01).

Some but not all authors have reported small but significant improvement in myocardial function, particularly in response to bone marrow-derived rather than pe-

ripheral blood mononuclear cells.<sup>101</sup> Homing studies suggest that only 1% to 3% of infused cells are retained in the heart, suggesting that the mechanisms underlying any benefit may not relate to myocyte regeneration.<sup>104</sup>

A number of small studies have assessed the value of peripheral injection of mononuclear cells harvested from the bone marrow or peripheral blood.<sup>105-109</sup> These studies have reported improvement in walking ability, healing of ulcers, peripheral tissue oxygen levels, endothelial-dependent relaxation, and angiographic appearance in patients receiving autologous mononuclear cells.<sup>106-109</sup> A small randomized trial in patients with non-reconstructable peripheral artery disease suggested that bone marrow-derived mononuclear cell injection or GCSF improved limb salvage.<sup>105</sup>

### Vascular repair

Because EPCs have been implicated in the normal physiology of vascular repair, mechanisms to promote them would be expected to reduce arterial disease. In a rabbit carotid balloon injury model, EPC transplant led to improved re-endothelialization and reduced intimal hyperplasia, particularly when endothelial nitric oxide synthase overexpression was induced.<sup>110,111</sup> GCSF administration before angioplasty in rats led to re-endothelialization and reduced intimal hyperplasia.<sup>53</sup> Therapy aimed at improving survival of EPCs, such as with statins, glitazones, and estrogen, are associated with reduced artery disease in animal models and patients.<sup>112-114</sup>

### Bioengineering of grafts and stents

EPC seeding and their mobilization has been reported to improve endothelialization of polytetrafluoroethylene grafts and stents in animal models.<sup>110,115</sup> Sin'oka et al<sup>116</sup> have reported the use of polymer tubes seeded with autologous bone marrow in children requiring pulmonary artery or vein replacement. They report excellent graft patency and that the diameter of grafts increases over time in keeping with recipient growth. Further studies are required to assess whether this approach might improve the patency of prosthetic grafts or stents.

Table II. Continued

No. of injections	High fat diet	Age of harvesting aorta	Atherosclerosis, * (mm <sup>2</sup> or %)		No. of injected cells per plaque
			Treatment	Control	
3	No	18 wks	450 ± 100 <sup>†</sup>	300 ± 20	3
3	No	18 wks	400 ± 100 <sup>†</sup>	300 ± 20	2
6 (every 2 wks)	Yes	14 wks	20% ± 1% <sup>†</sup>	35% ± 2%	Abundant (67%)
1	No	18 wks	185 ± 18 <sup>†</sup>	112 ± 13	Occasional
1	No	18 wks	110 ± 11	112 ± 13	Occasional

## CONCLUSIONS

A large number of studies support an association between bone and artery disease. The mechanisms underlying this association are starting to be understood and appear to include the involvement of bone marrow-derived cells in the normal repair and pathology of arteries. Further understanding of these mechanisms will be fundamental in attempts to utilize progenitor cells in therapy.

## AUTHOR CONTRIBUTIONS

Conception and design: JG  
 Analysis and interpretation: Not applicable  
 Data collection: JG, SP, AV, CR  
 Writing the article: JG, SP, AV, CR  
 Critical revision of the article: JG, SP, AV, CR  
 Final approval of the article: JG, SP, AV, CR  
 Statistical analysis: Not applicable  
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