The discovery of putative endothelial progenitor cells by Asahara and colleagues engendered a new era of vascular biology, full of the promise of using circulating and bone marrow–derived progenitors for therapeutic angiogenesis. In this landmark article, Asahara et al demonstrated that a CD34 or Flk-1 positive fraction of mononuclear cells isolated from human peripheral blood formed endothelial tube–like structures in vitro and incorporated into capillaries in regions of ischemic injury. Thus, a new paradigm of “vasculogenesis in the adult” was born. Since this observation, more than a decade has gone by, with more than 8000 articles published on endothelial progenitors. Despite this extraordinary productivity, therapeutic vasculogenesis with endothelial progenitor cells continues to be in its infancy—a hard luck story, dogged by conflicting studies, lack of uniform criteria for defining an endothelial progenitor, and nonrigorous methods of determining progenitor-mediated vasculogenesis.

Notwithstanding the fundamental problems plaguing this field, numerous studies during the past few years have suggested that vascular progenitors may be involved in maintaining vascular homeostasis and repair. The number of circulating endothelial progenitors is inversely related to cardiovascular risk, and patients with healthy vessels have a greater number of circulating endothelial progenitors. Coronary endothelial cells of extracardiac origin were observed in patients who had undergone gender-mismatched cardiac transplantation, and a similar robust degree of endothelial chimerism was seen in coronary vessels of patients with transplant arteriopathy. Interestingly, the degree of endothelial cells of extracardiac origin was almost log 10-fold higher in diseased segments of arteriopathic vessels than in nondiseased segments. These studies thus strengthened the notion that bone marrow and circulating progenitors were participants in atherosclerosis and arterial injury, although the precise functional role was unclear. The vasculoprotective nature of endothelial progenitor cells against atherosclerotic lesions was illustrated in an elegant study in which recurrent treatment with bone marrow–derived progenitors from young nonatherosclerotic ApoE−/− mice retarded progression of atherosclerosis in older ApoE−/− mice, despite persistent hypercholesterolemia. The authors demonstrated that bone marrow progenitors localized to atherosclerosis-prone regions of the vessel and concluded that bone marrow–derived progenitors helped decrease atherosclerotic burden by replacing senescent endothelial cells in plaques and by decreasing proinflammatory and proatherogenic cytokines. However, the initial enthusiasm associated with these studies has been tempered by other studies demonstrating the acceleration of atherosclerosis with transplantation of bone marrow–derived mononuclear cells.

In this issue of *Circulation*, Hagenson et al challenge the existing paradigm that bone marrow–derived progenitors contribute to the endothelium of plaques after arterial injury. The authors designed an elegant experimental approach wherein they determined the contribution of bone marrow cells to plaque endothelium or endothelial regeneration after mechanical plaque disruption. In a parallel series of experiments, the authors also investigated the contribution of circulating cells to endothelium in unruptured plaques or after plaque disruption. From the results of their experiments, the authors conclude that bone marrow–derived cells rarely contribute to the endothelium of developing plaques or participate in endothelial regeneration after plaque rupture. Second, using orthotopic arterial transplantation, the authors convincingly demonstrate that circulating cells rarely contribute to plaque endothelium or regeneration of overlying endothelium after plaque disruption. The experimental results strongly suggest that the local vessel is the major source of plaque endothelium and predominantly contributes to regeneration of overlying endothelium after plaque rupture.

So how do we interpret and reconcile this elegant study with the smorgasbord of studies previously published that lead us to very different conclusions? If bone marrow and endothelial progenitors do not contribute to the endothelium of plaques—either during plaque formation or after plaque rupture, as shown by Hagenson et al—then why is there a correlation between endothelial progenitor number, function, and vascular disease? If endothelial progenitors do not contribute to plaque endothelium, is there a strong scientific rationale to use them as therapeutic tools to improve vascular flow and retard atherosclerotic disease? Can we account for the major discrepancies in experimental results concerning the role and function of endothelial progenitors in vascular disease by technical reasons alone? Do seemingly minor differences in isolation of endothelial progenitor and culture...
methods used, genetic markers used to identify and trace their fate in tissues, technical methods of fixation, treatment of tissues, and microscopic analysis explain such widely different experimental findings?

It is likely that no single factor can be a major underpinning of such different experimental conclusions reached by so many different groups during the past decade. Rather, it is probable that a combination of differences in methodology, differences in biological properties of the cells being used, and differences in animal models underlie disparate observations that continue to plague a promising field. One of the biggest biological hurdles in this field of research has been to effectively compare, across multiple studies, the cell fraction being called “endothelial progenitors.”9 Many cell types have been labeled as endothelial progenitors; to complicate matters even further, endothelial progenitors are thought to exist in the local vessel wall as well. The term *endothelial progenitor* has been defined in so many different ways2 that the reader can be confused about the “progenitor properties” of an endothelial progenitor. Culturing bone marrow or circulating mononuclear cells to yield an endothelial-like “population” expressing characteristic endothelial surface markers and tube-forming capacity does not necessarily reflect isolation of progenitor cells but likely points to a heterogeneous collection of endothelial-like cells that may behave differently every time cell culture conditions are slightly modified. Thus, we have early endothelial progenitors and late endothelial progenitors, composed respectively of cells that are isolated within a few days (early) to a few weeks (late) of culture of circulating mononuclear cells. Early and late endothelial progenitors exhibit great differences in endothelial properties, including ability to form tubes, incorporation into vasculature in vivo, and secretion of growth factors. It is thus evident that experiments conducted with these 2 biologically different cell types can lead to completely different outcomes and conclusions. We thus propose that until a combination of cell types can lead to completely different outcomes and conclusions, studies should use multiple genetic markers driven by specific candidate promoters to better delineate the fate of endothelial progenitors. The tie2 promoter has been used extensively to follow the fate of endothelial progenitors, but tie2 is not a specific marker and is expressed by different nonendothelial cell types, including a monocytic/macrophage cell fraction. Studies using tie2 to identify bone marrow-derived endothelial cells can lead to confounding results, particularly after injury, inasmuch as many inflammatory cells at the site of injury may express tie2 without being committed to the endothelial pathway.10 Similarly, tracking of cells with endothelial-specific dyes should be interpreted with caution because many nonendothelial cell types, particularly those of the monocytic-macrophage lineage, also take up endothelial-specific dyes and can lead to erroneous interpretation of the fate of endothelial progenitors.

Although the article by Hagenson et al questions the role of bone marrow and circulating cells toward the contribution and regeneration of the endothelium in plaques, the experimental conclusions do not necessarily imply that endothelial progenitors or bone marrow–derived cells may not be useful for retarding atherosclerotic vascular disease. We must be open to alternative biological explanations such as paracrine effects rather than regeneration; injected bone marrow cells may alter the angiogenic milieu in the vessel wall and plaque, and this could lead to salutary effects on plaque size and atherosclerotic burden. Hagenson et al suggest that regeneration of plaque endothelium is likely from local endothelial cells or other progenitor cells residing within the local vessel wall. It is conceivable that injected endothelial progenitors could augment this reparative process without directly regenerating new endothelium. Such a salutary paracrine effect has been observed in myocardial infarction, wherein bone marrow–derived C-kit cells alter the local cardiac milieu to augment cardiac repair.11 Similarly, bone marrow–derived mesenchymal cells are known to exert cardioprotective effects through paracrine mechanisms.12

In summary, the elegant experimental approach by Hagenson et al reminds us of the inherent controversies with which the endothelial progenitor biology has been fraught. It is time that we pay attention to the precise type of cell used; the animal model considered; the fallacy of genetic markers; and alternative biological explanations, methods of analysis, and measurement of outcomes before we draw definitive conclusions. It is only by pursuing a rigorous, uncompromising approach, and adopting uniform criteria

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**Table. Recommendations About Future EPC Studies**

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<th>Recommendation</th>
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<tr>
<td>Describe clearly and precisely the phenotype of bone marrow cells used, particularly in studies determining functional effects in vivo. Avoid the term EPC. Instead, describe the precise phenotype of the cell used.</td>
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<tr>
<td>Recognize and understand that many genetic markers or dyes used for labeling and tracking EPC fates are often not entirely specific, and that there is a considerable overlap with cells of the monocoytic/macrophage lineage.</td>
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<tr>
<td>Ascribe high priority to studies that take the effort to explain discrepant findings. Granted, these studies may not be novel or groundbreaking but they will have a huge impact in moving the field in the right direction.</td>
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<tr>
<td>Assign greater emphasis into the mechanism of EPC action, recognizing that this may be different for different cell types considered. This is also critical for fine-tuning EPC therapy. We recommend that even clinical studies of EPC therapy use sophisticated imaging tools to better determine anatomical/structural changes in addition to functional changes in affected tissue.</td>
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EPC indicates endothelial progenitor cells.
for many of the above variables, that we as a scientific community can make significant inroads in this still-nascent field brimming with promise but dogged by a decade of hard luck (Table).

Disclosures
None.

References

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Arjun Deb and Cam Patterson

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