Cardiovascular-renal diseases are the leading causes of death and disability in the modern world. Moreover, chronic heart failure after myocardial infarction and peripheral arterial disease are predominant, devastating cardiovascular entities that limit prognosis and greatly impair quality of life in patients despite modern medical treatments. Cardiovascular diseases are also the predominant cause of death in people with renal diseases, underscoring the close relationship between these diseases. Until recently, medical efforts aimed only at prevention and slowing of functional deterioration after organ damage; however, the recent discovery of endogenous repair mechanisms involving hematopoietic stem and other progenitor cells has challenged the long-standing dogma regarding the inability to repair or regenerate terminally differentiated organs. A variety of stem and progenitor cell populations have shown properties that are potentially suited for tissue repair. After encouraging results in preclinical animal models, early clinical studies of endothelial progenitor cells (EPC) have begun. Envisioning the goal of true tissue regeneration, application of bone marrow–derived progenitors for heart and limb ischemia has provided early evidence of safety and feasibility. These studies have provided data indicating functional improvement as well. Although there is strong experimental and clinical evidence for a regenerative function of EPC even in renal disease, there has been no clinical application of this approach. After the initial flurry of activity, it is time to reconsider these approaches and attempt to optimize functional improvement and patient safety. This article briefly recapitulates biologic and functional characteristics of EPC before giving a concise overview on current therapeutic EPC applications in the field of cardiovascular-renal medicine with consideration of future challenges.
dened by exceptionally high cardiovascular morbidity and mortality.\textsuperscript{6,7} They predominantly die of arteriosclerotic diseases such as myocardial infarction and stroke.\textsuperscript{6}

Within recent years, evolving research has challenged the classical dogma that terminally differentiated organs lack the capacity for cell turnover and regeneration. The recent discovery of endogenous repair mechanisms implicating hematopoietic stem and progenitor cells set off the concept of tissue repair. By definition, stem cells (SC) possess the capability of self-renewal, transformation into dedicated progenitor cells, and differentiation into specialized progeny such as endothelial progenitor cells (EPC).

Theoretically, a variety of SC and progenitor cell populations possess properties that are suited to tissue repair. Apart from EPC, mesenchymal, embryonal, resident cardiac, embryonic stem cells and skeletal myoblasts are considered, respectively. Each population brings its own profile of advantages, disadvantages, and, most important, practical limitations in the clinical setting. Because comparative evaluations of cell populations remain scarce, some investigators applied unFractionated bone marrow (BM) cells (BMC) containing various stem and progenitor populations, including hematopoietic SC and EPC. This review focuses on the regenerative potential of EPC. We briefly recapitulate the biologic characteristics and functional cascade of EPC from mobilization out of the BM to their migration and eventual functioning at the site of ischemia. We consider current therapeutic applications of EPC in the field of cardiovascular-renal medicine in regard to technical aspects and future challenges.

EPC

Cellular Biology

In contrast to SC, progenitor cells are immature cells with a more limited differentiation potential. They proliferate for a finite number of cell divisions.\textsuperscript{7–10} By convention, EPC are characterized by hematopoietic SC markers such as CD34 or CD133 and expression of an endothelial surface marker (vascular endothelial growth factor receptor-2 or kdr, von Willebrand factor, VE cadherin, CD146), uptake of Dil-acetylated lipoprotein, and lectin binding.\textsuperscript{11} On the basis of surface markers, EPC can be quantified and isolated by flow cytometry. Because of their adhesion properties to fibronectin, EPC can be further grown in culture from unFractionated BMC using selectively enriched medium.

EPC that were originally isolated from the mononuclear cell fraction of peripheral blood\textsuperscript{12} have subsequently also been isolated from human umbilical cord blood as well as from mononuclear cell, CD34\textsuperscript{+} and CD133\textsuperscript{+} hematopoietic SC fraction derived from BM. It seems that the BM may not be the single source of EPC, because a CD14\textsuperscript{+}/CD34\textsuperscript{+}/CD133\textsuperscript{+} mononuclear side population with angiogenic profile has been characterized in various tissues.\textsuperscript{11} Overall, controversy still exists regarding the identification and origin of EPC.\textsuperscript{11}

Mobilization and Homing of EPC

Whereas EPC levels remain low under normal conditions,\textsuperscript{12} ischemia of limb muscle or myocardium is believed to be the predominant trigger for EPC to travel from the BM niche. The release of EPC from BM is regulated by a variety of growth factors, enzymes, ligands, and surface markers. Today, the concept of EPC mobilization envisions an ischemia-dependent secretion of proangiogenic cytokines as stromal cell–derived factor-1 (SDF-1)\textsuperscript{13–15} or vascular endothelial growth factor (VEGF)\textsuperscript{16,17} induced by hypoxia-inductive factor at the site of ischemia, which, in turn, are released to the circulation.\textsuperscript{14} Other EPC-mobilizing factors are placental growth factor, G-CSF, and GM-CSF. In the BM, however, activation of matrix metalloproteinase-9, which cleaves membrane-bound Kit ligand to a soluble Kit ligand, releases cKit\textsuperscript{+} EPC to the vascular zone of the BM niche.\textsuperscript{18} Endothelial nitric oxide synthase–derived nitric oxide is a prerequisite for matrix metalloproteinase-9.\textsuperscript{19}

Hetero-, homo-, and autologous EPC are capable of entering sites of active neovascularization. Asahara et al.\textsuperscript{17} detected donor-derived EPC in the myocardium after myocardial infarction. This observation provided the first support of the concept that ischemia-mobilized EPC invade ischemic tissue and participate in postnatal angiogenesis (homing). Recruitment of EPC requires a coordinated sequence of multistep adhesive and signaling events, including adhesion, chemotraction, and migration. Integrins and tissue-specific adhesion molecules are key elements for adhesion of hematopoietic SC.\textsuperscript{20} Angiogenic chemokines such as SDF-1 and VEGF do not merely function systemically to mobilize EPC from the remote BM. These factors also attract circulating EPC locally to the ischemic tissue, as we and others have shown. SDF-1 overexpression enhances homing of EPC\textsuperscript{21} that are particularly sensitive to SDF-1.\textsuperscript{22} It is interesting that the migratory capacity of EPC toward SDF-1 and VEGF determines functional improvement of postinfarct patients.\textsuperscript{23}

Regenerative Mechanisms

For a long time, researchers believed the vascular endothelium to be an inactive, inner lining of blood vessels. Repair of the endothelium was believed to occur by proliferation of endothelial cells from wound margin to center. However, in recent years, the endothelium has been identified as a highly active structure running from the endothelial layers of conduit vessels through into microcirculation. In addition to providing lining, the endothelium provides essential functions. Moreover, the disease process of “dysfunctional” endothelium is closely related to arteriosclerosis and cardiovascular disease.\textsuperscript{24,25} An even more novel perspective proposes an interaction of BMC and EPC with the endothelium to repair vasculature by angiogenic action.\textsuperscript{26} Shi et al.\textsuperscript{27} also observed a rapid coverage of implanted grafts by CD34\textsuperscript{+} BMC (engraftment).

Angiogenesis is crucial for preservation of tissue function in critical ischemia. This mechanism is driven by ischemia and represents growth of new capillaries by sprouting and intussusception from preexisting capillaries. The mechanism is clearly distinct from arte-
riogenesis, the collateral growth from conduit arteries driven by biomechanical forces. Asahara et al. first linked EPC to postnatal angiogenesis in their seminal study. EPC have further been shown to promote repair of endothelium (reendothelialization) and postnatal formation of new capillaries (neovascularization). This finding has been reinforced by animal models of hind-limb ischemia and myocardial infarction, where progenitor cells induced new vessel formation.

Earlier, EPC were believed to affect vascular repair by differentiation into endothelial cells. At first, tracking of engrafted cells initially revealed the expression of cardiac and/or endothelial marker proteins pointing to a tissue-specific differentiation of transplanted cells. Subsequently, the observation of SC transdifferentiation was more accurately explained as cell–cell fusion of transplanted and local cells. Consistent with the idea of transdifferentiation, EPC historically were reported to incorporate vessel structure. In line with other investigators, Ziegelhoeffer et al. questioned the hypothesis, observing no incorporation of BMC into growing vessels structure while BMC accumulated in perivascular areas. Hence, the capability to promote vascular growth seemed to be related to paracrine effects. Homing of EPC to the ischemic tissue releases cytokines, chemokines, and growth factors. These secreted factors, in turn, affect the surrounding ischemic tissue in a paracrine manner. BM-derived mononuclear cells have been reported to release multiple angiogenic cytokines such as VEGF, angiopoietins, and fibroblast growth factor. Angiogenic factors have originally been discovered in the regulation of tumor vascularization. EPC promote angiogenesis, suppress cell death of resident cells, and recruit SC by factor release. Because SC and progenitor cells might serve more as a “cytokine factory” rather than the building material, the external EPC application potentially boosts endogenous repair mechanisms after ischemia occurs. Therefore, cell therapy at this time seems more favorable than a single-gene therapy.

Preclinical Experiences
Driven by a multitude of experimental findings that have elevated the role EPC in tissue repair, we and others have extensively studied EPC in animal models of ischemic hind-limb and myocardium. Of interest in our study, mononuclear cells that were depleted of CD34+ cells failed to improve myocardial function after infarction, suggesting that the CD34+ population plays an important role in regeneration by BMC transplant. Systemic application of expanded EPC and selective application of purified CD34+ cells or mononuclear BMC improved myocardial function. In another preclinical animal study, we observed functional improvement in chronic myocardial ischemia. Although the underlying mechanisms require further definition, the regenerative characteristics of EPC provide a highly attractive therapeutic option.

CLINICAL IMPLICATIONS OF EPC

EPC and Cardiovascular Disease
EPC correlate well with risk factors of atherosclerosis; age, smoking, hypercholesterolemia, and diabetes affect EPC number and function. Manifest coronary artery disease, angina pectoris, and myocardial infarction have a robust impact on EPC mobilization. In particular, unstable angina pectoris and acute myocardial infarction augment EPC levels. EPC levels are inversely related to the severity of congestive heart failure. Conversely, physical activity enhances EPC levels and activity. Statins, an essential in gold-standard treatment of cardiovascular disease, revive EPC levels by activation of the Akt/phosphatidylinositol-3 kinase pathway. Other vasoprotective drugs, such as angiotensin receptor blockers, show similar effects. Overall, the biology of EPC is strongly related to the cardiovascular risk burden in healthy and manifest individuals.

EPC and Renal Disease
Comparatively little is known about EPC for renal disease treatment. Functional recovery after toxic or ischemic kidney mainly comes along with proliferation and replacement of tubular cells. Endothelial and tubular cells have a remarkable capacity to recover. Although the underlying mechanisms are not fully understood, replacement of damaged cells will mainly be generated by neighboring cells or cells recruited from the circulation. Although the origin of newly generated renal cells remains to be determined, it has been observed that BMC can repopulate nephrons after ischemia. Therefore, the role of progenitor cells in treating renal disease deserves greater attention.

In the clinical setting, renal diseases in concert with cardiovascular risk factors significantly influence the number and function of EPC. Advanced renal failure, dialysis, and renal transplantation impair EPC capacity. Adverse findings of elevated EPC in some studies might be explained by concurring drug therapy, for example, erythropoietin (EPO). Uremia has further been described to alter EPC function as to differentiation, mobilization, migration, and tube formation. In a previous study, fewer CD34+ and AC133+ cells were circulating in patients undergoing dialysis compared with a control group. More recently, Westerweel et al. found the balance between vasoprotective EPC and adverse smooth muscle progenitor cells adversely shifted to a state of impaired vascular repair in patients with end-stage renal disease. Similarly, chronic renal disease tends to impair EPC, even though it is barely possible to separate the potential effect from confounders.

Conversely, support or replacement of renal function seems to be accompanied by recovery of EPC biology. Within 12 mo after kidney transplantation, uremia-induced EPC function was restored in a small sample population, although EPC numbers remained unchanged. In line with this finding, already nocturnal hemodialysis improved impaired EPC levels and function in patients with end-stage renal disease.
whereas in the conventional hemodialysis group, EPC biology was impaired. Speculatively speaking, this finding might be explained by a poorer uremic clearance from intermittent conventional dialysis.\textsuperscript{81} EPO, an important hematopoietic factor that is deficient in chronic renal disease, has also been shown to affect EPC. EPO plasma levels are independently related to EPC levels.\textsuperscript{82} In vivo studies showed that application of recombinant EPO, in turn, augments EPC levels in the spleen and peripheral blood by activating the Akt kinase pathway.\textsuperscript{83}

Referring to their regenerative properties, EPC seem to support repairing mechanisms in kidneys of endothelial, mesangial, and tubular structures. Transplantation of EPC expanded from a muscle SC pool, locally engrafted, and improved renal function in an acute renal ischemia rat model.\textsuperscript{84} If the rate of glomerular cell renewal under normal conditions averages approximately 1% per day,\textsuperscript{85} the rate is accelerated after injury. Some animal studies provide evidence that EPC contribute to glomerular capillary repair.\textsuperscript{86,87} In humans, acceptor-derived EPC have been localized in kidney allografts, replacing donor endothelial cells and potentially repairing transplant-related vascular injury.\textsuperscript{88} In a glomerulonephritis rat model, injection of BM-derived mononuclear cells into the renal artery improved endothelial injury and mesangial cell activation.\textsuperscript{89} BM-derived progenitors are involved in the turnover and repair of mesangium\textsuperscript{82,90,91} and tubules, which are highly regenerable.\textsuperscript{92,93}

Overall, these findings strongly advocate that EPC can also play a significant role in renal repair and present an exciting target for regeneration in renal disease.

**THERAPEUTIC APPLICATIONS**

Apart from ideal cell type, the route of cell delivery is another critical question. Basically, the goal of cell transplantation is to selectively deliver an optimal number of the most effective cells to the region of interest without safety concerns and via a minimally invasive route. It follows that cell retention, the fraction of cells that are retained in the transplant site over time, and local milieu, which determines cell fate, are relevant. In case of transvascular applications, even endothelial cell adhesion, vessel wall transmigration and tissue invasion are variables of transplant efficiency.

**Routes of Delivery: Transvascular versus Direct Application**

Taking the lead from selective coronary artery intervention, cell transplantation after myocardial infarction is delicately performed through a balloon-tipped catheter by small access. For optimization of the contact time of cells and coronary microcirculation, cell infusion will be performed while the inflated balloon blocks cell reflux.\textsuperscript{94} In addition, this transvascular cell therapy is influenced by the secretion of chemoattractive factors from the ischemic tissue after a recent infarction or in recurring ischemia.\textsuperscript{95} In contrast, even though intravenous infusion of EPC seemed to have some effect on cardiac function after infarction,\textsuperscript{96} unselective tissue homing to nontarget organs limits efficiency using this approach.\textsuperscript{97} A comparative study of both approaches confirmed only cell homing after the transvascular method of infusion.\textsuperscript{98}

In the case of chronic infarction, a transvascular strategy might be impaired by occlusion of the afferent artery. Abated chemoattraction after a previous infarction will further impede the transvascular approach. Direct cell injection into ischemic tissue overcomes these impairments and, therefore, seems the more suitable approach in the case of chronic infarction. With regard to the heart, direct injections have been performed transepicardially and transendocardially. On the downside, direct injection causes cell cluster in malperfused tissue, resulting in poor cell survival, whereas conceptually, transvascular cell transfer should distribute cells more homogeneously. Conversely, direct application allows for the delivery of larger cells such as myoblasts, which cannot be applied transvascularly by virtue of microembolization. Direct injection, which is even more invasive, because in terms of the heart it requires direct access, can also be considered. The more invasive the techniques, the higher are the periprocedural risks for patients who already have cardiovascular-renal disease. Last but not least, mechanical puncture of necrotic myocardium comes with an inevitable risk for perforation and ventricular rupture. This, however, is not true for leg ischemia.

Both SC and progenitor cells can be mobilized from their home in BM into the peripheral circulation using a cytokine. This noninvasive strategy is borrowed from hematologists who isolate SC before BM is irradiated for SC transplantation. The systemic applications of the potent cytokine SC-mobilizing agents SC factor and G-CSF also improved cardiac function after infarction in animals.\textsuperscript{35,99} Thus, it seems systemic application of mobilizing factors has the potential to augment EPC-mediated repair mechanisms safely and effectively.

**Myocardial Disease**

**Myocardial Infarction**

Soon after experimental data suggested a functional improvement in cardiac function by using cells from BM after infarction,\textsuperscript{31,37,54,55} human studies were done by infusing selected or unselected BMC into the artery that was blocked, in addition to traditional treatment. Initially, three smaller, uncontrolled studies\textsuperscript{94,100,101} suggested feasibility of intracoronary application of BMC in humans. Since those earlier attempts, a couple of larger, randomized, partly placebo-controlled trials have been completed. In the randomized, controlled Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) and Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) trials, global left ventricular (LV) function was improved 4 to 6 mo after cell transfer, although LV dimensions remained unchanged.\textsuperscript{102,103} These promising studies were opposed by two negative trials that had some relevant differences in study
design, even though the exact reasons for the conflicting results remain elusive. For example, Janssens et al.104 infused BMC a full day after the infarction when acute coronary reperfusion had already occurred. In the other study, the Autologous Stem Cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial, the baseline magnetic resonance imaging was not performed until 2 to 3 wk after the cell transfer. Cells were administered late and, of greater concern, prepared differently.105 However, both studies failed to show an improvement of LV function or dimension 4 and 6 mo, respectively, after cell transfer. In longer follow-up of REPAIR-AMI, the improvement of LV function remained stable after 12 mo,106 whereas the 6-mo effects were no longer present at 18 mo of follow-up of the BOOST collective.107 However, looking at the studies overall suggests that the infusion of BMC into the once blocked, recanalized artery is both safe and efficient. No adverse events, such as in-stent stenosis, stent thrombosis, ventricular arrhythmias, myocardial calcifications, or tumor formation were reported during the period from infusion to 18 mo afterward. Double-blind, large-scale, randomized, placebo-controlled, multicenter trials are under design to investigate further these promising results.

The strategy to mobilize progenitor cells after myocardial infarction by administering G-CSF is highly debated. The much criticized Myocardial Regeneration and Angiogenesis in Myocardial Infarction With G-CSF (MAGIC) pilot study revealed an increased risk for in-stent restenosis, apart from its problematic design.108 G-CSF–induced cell mobilization performed shortly after an acute angioplasty in acute infarct improved LV function and metabolic activity at the cost of serious adverse effects.109 Another study with a more favorable risk profile did not show a beneficial effect on LV function.110 All in all, we strongly believe that the safety of G-CSF application for cell mobilization in myocardial infarction must be studied further.

**Intractable Coronary Artery Disease**

Some patients with diffuse coronary artery disease and without any option of revascularization have intractable angina despite optimal medical treatment. Recurrent myocardial ischemia can further deteriorate already poor cardiac function by hibernation of viable myocardium. In this therapy–refractory, highly compromised population, cell therapy offers the single potential to relieve symptoms as well as improve quality of life by augmenting regional perfusion; there is nothing else to offer them. SC and progenitor cells seem to deliver a cocktail of angiogenic cytokines to ischemic tissue.46 In a set of small-scale studies, patients received direct injection of unfractionated BMC transendocardially, during open-heart surgery,111 or transendocardially, under electromechanical guidance.52,112,113 Overall, both approaches seemed safe and feasible. Pathology, physical capacity, and ventricular function were reported to improve. Small patient numbers and uncontrolled study designs, however, do not allow final conclusions regarding treatment efficacy. Therefore, larger, randomized, controlled studies are needed. It is interesting that the strategy of noninvasive pharmacologic mobilization of EPC by G-CSF did not improve myocardial function, while effectively increasing circulating EPC. In addition and of great concern, myocardial reinfarction associated to G-CSF therapy was reported.114 Because harvesting and infusing mobilized cells to chronically infarcted tissue remains uncertain, it cannot be promoted.

**Chronic Heart Failure and Ischemic Cardiomyopathy**

Encouraged by the results in its use for myocardial infarction, cell therapy was offered to patients with chronic heart failure after previous infarction. In the initial study, CD133+ cells were directly injected into the infarct boarder zone during a bypass procedure in patients who had had previous myocardial infarction. Because no adverse events were reported, treatment was considered safe and feasible. The finding of improved LV function is limited by the lack of a control group.115,116 In another study, unfractionated BMC were injected transvascularly. The follow-up did not reveal any safety concerns while significantly increasing LV function compared with the control groups.117 The most comprehensive study on this group was recently published by Assmus et al.118 In a carefully designed crossover study, the authors observed a significant improvement of ventricular function 3 mo after transvascular application of BM-derived progenitor cells in patients with resolved myocardial infarction.

**Peripheral Arterial Disease**

So far, only a single study has used cell therapy in peripheral vascular artery disease. In the randomized, placebo-controlled Therapeutic Angiogenesis using Cell Transplantation (TACT) study, symptoms and parameters of leg ischemia were alleviated after the injection of BMC by inducing neovascularization.119 Further studies are need to underscore this single-study finding.

**Renal Disease**

The complex structure of kidneys, with their arterioles, capillaries, glomeruli, and tubules interacting in a three-dimensional structure to provide the complex function of filtration and reabsorption, complicates regenerative approaches. Because renal tissue was believed to be terminally differentiated, regeneration once renal damage has occurred seemed to be impossible. Encouraged by promising experimental findings as described in the cardiovascular system, a clinical approach seems to be worth further study. To our knowledge, no investigation of cell therapy to promote kidney repair has been published but should come soon; however, referring to the implications of EPO and EPC, it is of particular interest that the application of recombinant EPO improved EPC levels and functions in a pilot study.120

**FUTURE PERSPECTIVES**

The application of EPC seems to improve tissue perfusion and function after
ischemia. After the initial flurry of early clinical studies, it is time to reconsider its real potential. In order to advance regenerative medicine, deeper knowledge of the underlying mechanism in this treatment is mandatory. The disappointing regional retention of transplanted cells at this time, for example, needs improvement. In the clinical setting, cell type, dosage, and timing of application need further refinement. Even though the clinical application seemed to be safe over a span of months, long-term follow-ups for adverse events, morbidity, mortality, and quality of life are essential before final safety conclusions can be drawn. Overall, conducting large-scale, double-blinded, randomized, controlled studies is the only way to find the answers to remaining questions and ensure patient safety and improved outcomes.

Regardless of detailed mechanisms, we and others truly believe that regenerative cell therapy offers the potential to reestablish perfusion and regain function of injured organs by means of neovascularization and secretion of proangiogenic factors in cardiovascular-renal disease.121–123 Early experiences of clinical applications in the field of renal disease should follow the promising results realized in applying this method for cardiac disease very soon. Depending on further molecular insights, we envision a combination, multitest approach including SC and progenitor cells, multiple, precisely timed factors, and even more to lift regenerative medicine to the next level.

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DISCLOSURES

None.

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