Peripheral artery disease (PAD) refers to noncoronary vascular disease affecting the peripheral arteries. Most commonly the term is applied to occlusive arterial disease affecting the limb arteries, typically due to atherosclerosis. Preclinical studies indicate that a variety of stem cell therapies provide growth factors and cytokines for therapeutic angiogenesis. Small clinical trials with bone marrow mononuclear cells, as well as other cell types, have shown promise. However, mechanisms of therapeutic effect, if any, are not understood. Definitive clinical trials are needed to determine if there are any beneficial effects on functional capacity or morbidity.

Abstract

Peripheral artery disease (PAD) refers to noncoronary vascular disease affecting the peripheral arteries. Most commonly the term is applied to those individuals with lower extremity disease. More than 8 million people in the United States suffer from arterial occlusive disease affecting the lower extremities, usually due to atherosclerosis. PAD shares causative risk factors with coronary arterial disease (CAD) including smoking, diabetes mellitus, hypertension, hypercholesterolemia, hyperhomocysteinemia, elevated fibrinogen, and elevated C-reactive protein. In addition, beta-2 microglobulin, cystatin C, and C-reactive peptide are elevated to a greater degree in PAD than in CAD.1,2 PAD is an increasingly important public health issue in the United States because of persistent tobacco usage and the anticipated rise in diabetes prevalence.3

An office-based diagnosis of PAD can be made by measuring the blood pressure at the ankle and the arm to calculate an ankle-brachial index (ABI). This measurement is generally made using a hand-held Doppler, because Korotkoff sounds are not audible with a stethoscope in the lower extremities. The normal ABI is 1.0 to 1.2, and PAD is diagnosed when the ABI falls below 0.9. In at-risk individuals (i.e., <70 years, or <50 years with a history of diabetes mellitus or tobacco use), the risk of PAD as detected by ABI is as much as 29%.4 Individuals with advanced age or long-standing diabetes may have fibrocalcific disease of the pedal arteries that causes an artifactual elevation of the ABI. Toe pressures and pulse waveform recordings can detect PAD in these individuals.

The disease spectrum of PAD varies from asymptomatic to intermittent claudication and critical limb ischemia, which may lead to gangrene and amputation. Based on symptoms and clinical signs, two clinical classifications (Fontaine5 and Rutherford6) are used to measure the severity of PAD. In the early stages of PAD, patients may not report symptoms. As PAD progresses, intermittent claudication occurs and patients experience pain in the leg muscles when walking. More advanced disease causes pain at rest, typically occurring in the foot at night. This limb is in jeopardy, and any injury can lead to cutaneous ulcerations, gangrene, or limb loss. Furthermore, the risk for cardiovascular mortality becomes greater as the severity of PAD increases.

PAD causes marked derangement in vascular homeostasis, e.g., impaired synthesis and bioactivity of vasodilator factors such as nitric oxide; increased generation of oxygen-derived free radicals; increased expression of adhesion molecules (VCAM-1) and chemokines (e.g., MCP-1) mediating vascular inflammation; and a shift toward prothrombotic factors such as PAI-1.7 Hallmarks of human endothelial senescence include attenuation of proliferative capacity, reduced ability to align with shear stress, and shortened telomeres—most notably at bifurcations, where endothelial cell (EC) turnover is increased. A severe and systemic vascular dysfunction in these individuals places them at risk of stroke, myocardial infarction (MI), and limb loss. The vascular derangement is attributed to cardiovascular risk factors such as diabetes mellitus, hypercholesterolemia, hypertension, and smoking as well as sedentary state and the Western diet. Modification of risk factors—with statins, converting enzyme inhibitors, insulin sensitizers, tobacco cessation, exercise, and diet—reduces morbidity and mortality. However, medical therapies are ineffective at increasing limb perfusion, and interventional approaches are costly and often short-lived.

Vascular Regeneration in PAD

Therapeutic angiogenesis is an experimental approach to restore perfusion. We and others have shown that angiogenic growth factors improve limb perfusion in preclinical models,8 but clinical trials (i.e., with VEGF, FGF, or HIF-1α) have shown minimal or no benefit.9,10 By contrast, cell therapy may provide an ongoing source of growth factors and structural elements for therapeutic angiogenesis. The use of therapeutic cells for cardiovascular regeneration was presaged by Asahara’s discovery in 1997 of the vasculogenic “endothelial progenitor cell” (EPC) subpopulation11 and has culminated in small randomized clinical trials that show promise. Therapeutic cells for cardiovascular regeneration, whether harvested from the marrow, blood, or tissue, share a number of traits that make them appealing as candidates for cell-based...
regeneration. The first is that these cells do not need to overcome an immunologic barrier. Also, this approach is not burdened by the ethical concerns that surround the use of human embryos. Furthermore, the trials done to date show little increased risk associated with their use in humans.

Mechanisms by which cell-based therapies improve tissue perfusion are not well defined, and the bone marrow-derived mononuclear cells (BMMNCs) or peripheral blood mononuclear cells (PBMCs) utilized for cardiovascular trials are a mixed population of cells that have not been completely characterized. Within this population of cells, there are true endothelial progenitors that can incorporate into the vascular network. These cells are rare, only 1 to 2 per 100 million mononuclear cells. The surface markers that are commonly used for identifying human EPCs include CD133, CD34, and KDR (kinase insert domain receptor, or vascular endothelial growth factor receptor 2), but these markers are not unique to EPCs. At present, the best approach currently to distinguish early endothelial progenitors is to define endothelial lineage functionally (i.e., by the ability of true endothelial progenitor cells to inosculate into a pre-existing vascular network). In cell culture, late-outgrowth colonies that grow in a cobblestone pattern are reminiscent of endothelial cells (ECs). However, cells derived from EPC early outgrowth colonies express similar surface markers (CD31, CD105, CD144, CD146, von Willebrand factor, KDR, and UEA-1) and incorporate acetylated low-density lipoprotein, but they also express the myeloid surface markers CD45 and CD14 and have other features of the monocyte/macrophage phenotype. These cells clearly are not of endothelial origin and express markers of hematopoietic lineage; however, some of these cells may contribute to angiogenesis by secreting angiogenic cytokines and matrix metalloproteinases. Additionally, other bone marrow-derived cells, such as pericytes, can associate with and stabilize endothelial networks.

It is possible that a greater effect and/or more persistent benefit of cell therapy could be realized with a more precisely defined cell population. Additionally, it is not known what combination of progenitor cells might be most therapeutic in human, e.g., purified EPCs or some combination with smooth muscle or other precursors and/or subsets of hematopoietic progenitors. Thus, greater clarity is needed regarding the composition and fate of the cells that are used for cardiovascular cell therapy. Improved characterization of the administered cells could provide insights into which subsets might be associated with these physiological processes and with improvements in clinical endpoints.

Cell Therapy in PAD: Where Do We Stand

The focus of cell-based therapy in PAD has primarily been on progenitor cells derived from bone marrow or peripheral blood, and other cell types are entering clinical trials including mesenchymal stromal cells derived from placenta. The goal of this therapy is to promote collateral vessel formation that will improve blood flow to ischemic tissue, thereby alleviating symptoms and, in severe cases, enhancing limb preservation. The first clinical trial of cell therapy for PAD was performed by Tateishi-Yuyama et al. in 2002 (Therapeutic Angiogenesis using Cell Transplantation Study, or TACT)23. In this study, injection of BMMNCs into the gastrocnemius muscle of patients with diabetes and symptomatic PAD resulted in an improvement in ABI, transcutaneous oxygen tension, pain-free walking time, and rest pain. Since that time, numerous other studies have examined the efficacy and safety of cell therapy for PAD, with varying results (Tables 1 and 2).22-35

Most clinical trials of cell therapy for PAD have consisted of uncontrolled patient series, with few randomized, properly controlled studies. Sample sizes have been relatively small, with most studies enrolling fewer than 50 patients. Varying degrees of PAD severity have been included, ranging from intermittent claudicants to patients with critical limb ischemia. The therapeutic product for nearly all of the trials has been bone marrow-derived mononuclear cells and/or peripheral blood-derived mononuclear cells (PBMCN) harvested with or without granulocyte colony-stimulating factor mobilization. Cells were delivered by direct intramuscular injection at multiple sites of the affected limb or by intra-arterial injection via the femoral artery. Reported endpoints of these studies have included ABI, transcutaneous oxygen tension, and angiography examined at baseline and following cell therapy, with an average follow-up period of approximately 6 months to 1 year. Subjective outcomes have also been reported, including patient-perceived rest pain and pain-free walking time or distance.

Collectively, results from these studies, including ulcer healing and limb salvage, are encouraging, but the number of patients studied is small in most trials (Tables 1 and 2).22-33 Procedures associated with cell therapy, such as bone marrow aspiration and injection, were generally well-tolerated by the patients, with few adverse events reported. Hence, current data is promising, and safety concerns do not outweigh the promise of potential benefit, which must now be tested in definitive clinical trials.

Cell Therapy in PAD: Where Do We Go From Here

Major criticisms of current studies focus on the relatively small sample sizes, lack of randomization and proper controls, insufficient characterization of the cell product, and inadequate knowledge regarding the route and site of administration, dosing, duration of therapy, survival of the cells, and their mechanism(s) of action.

A noticeable deficiency in our current studies is an understanding of the mechanism(s) underlying the potential beneficial effect(s) of cell therapy in treating PAD. Additionally, there are few studies that characterize the cellular/molecular properties of the therapeutic product used in the trials. These data would greatly enhance our understanding of how the effect(s) of cell therapy are being attained and would facilitate optimization of therapy and design of future clinical trials.

The Cardiovascular Cell Therapy Research Network, which was established by the National Heart, Lung and Blood Institute, has designed the PACE (Patients with Intermittent Claudication Injected with ALDH Bright Cells) study to overcome such limitations. The randomized, double-blind, placebo-controlled clinical trial will enroll 80 patients with symptomatic intermittent claudication. This study will assess the safety and efficacy of autologous bone marrow-derived ALDH bright cells. Preclinical studies have demonstrated that the ALDH bright cells have angiogenic activities possibly through the release of angiogenic factors,34,35 and small pilot studies in patients with critical limb ischemia or heart failure have suggested that their administration is safe.36,37 A novel cell characterization approach that we hope to apply to this and future studies is mass cytometry.38 This technology permits single-cell analysis of up to 100 surface markers and intracellular parameters to identify specific cell subsets that correlate with clinical endpoints and provide predictors for therapeutic success.
edge technology in imaging to track cells and visualize their effects on the treated tissue, and continue their efforts to deeply characterize the cell products and delineate mechanisms of action. Nevertheless, we are optimistic that therapeutic enhancement of vascular regeneration is an achievable goal.

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### Conclusion

New therapies for PAD are needed, and preclinical studies suggest that stem cell therapies may be useful in treating vascular disease. Since the TACT study was published in 2002, there have been more than 30 reported therapeutic cell trials in patients with PAD. However, the majority of these studies are uncontrolled and small. Although these studies suggest that cell therapy is safe and feasible, many of them failed to reach their primary therapeutic endpoint. Furthermore, many questions remain regarding the type of cell (or combination of cells) to utilize, the dosage, the duration, and the route of administration. For the promise of stem cell therapy to be realized, clinical investigators must perform carefully controlled trials that are adequately powered, incorporate cutting-edge technology in imaging to track cells and visualize their effects on the treated tissue, and continue their efforts to deeply characterize the cell products and delineate mechanisms of action. Nevertheless, we are optimistic that therapeutic enhancement of vascular regeneration is an achievable goal.

### Table 1. Controlled PAD cell therapy clinical trials. BMMNC: bone marrow-derived mononuclear cell; G-CSF: granulocyte-colony stimulating factor; PBMNC: peripheral blood-derived mononuclear cell; PAD: peripheral artery disease

| Reference            | Number of Patients | Number of Control Patinets | Type of Cell                        | Follow-up       |
|----------------------|--------------------|-----------------------------|-------------------------------------|-----------------|
| Tateishi-Yuyama21    | 22                 | 24 limbs                    | BMMNC or BMMNC and PBMNC           | 4 and 24 weeks  |
| Huang22              | 14                 | 14                          | G-CSF mobilized PBMNC              | 3 months        |
| Arai23               | 13                 | 12                          | BMMNC                              | 1 month         |
| Bartsch24            | 13                 | 12                          | BMMNC                              | 2 and 13 months |
| Corbellis25          | 10                 | 9                           | BMMNC                              | 12 months       |
| Prochazka26          | 42                 | 54                          | BMMNC                              | 4 months        |
| Walter27             | 19                 | 21                          | BMMNC                              | 3 and 6 months  |
| Iafri28              | 34                 | 12                          | BMMNC                              | 1, 4, 8, 12 weeks|
| Idei29               | 51                 | 46                          | BMMNC                              | 3-4 years       |
| Benoit30             | 34                 | 14                          | BMMNC                              | 6 months        |
| Powell31             | 32                 | 14                          | Expanded BMMNC                     | 6 months        |
| Powell32             | 48                 | 24                          | Expanded, multicellular therapy    | 12 months       |
| Losordo33            | 16                 | 12                          | Enriched CD34+ BMMNC               | 6 and 12 months |

### Table 2. Outcomes for PAD cell therapy clinical trials. ABI: ankle-brachial index; TcPO2: transcutaneous oxygen tension.

| Reference            | ABI    | TcPO2 | Limb salvage |
|----------------------|--------|-------|--------------|
| Tateishi-Yuyama21    | Improved | Improved | Improved     |
| Huang22              | Improved | Improved | Improved     |
| Arai23               | Improved | Improved | Improved     |
| Bartsch24            | Improved | Improved | Improved     |
| Corbellis25          | Improved | Improved | Improved     |
| Prochazka26          | Improved | No Effect | Improved     |
| Walter27             | No Effect | Improved | Improved     |
| Iafri28              | Improved | Improved | Improved     |
| Idei29               | Improved | Improved | Improved     |
| Benoit30             | No Effect | Improved | Improved     |
| Powell31             | No Effect | Improved | Improved     |
| Powell32             | Improved | Improved | Improved     |
| Losordo33            | No Effect | Improved | Improved     |
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