Peripheral Arterial Disease

Medical Therapy in Peripheral Artery Disease

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The epidemiology of peripheral artery disease (PAD) is well described and related to age and, in particular, the risk factors of diabetes mellitus and smoking. Recent data from the National Health and Nutrition Examination Survey found a 5.9% prevalence in subjects ≥40 years of age resulting in an estimated prevalence of 7.2 million affected individuals in the United States. These subjects have a significant increased risk of all-cause mortality because of the underlying atherosclerotic disease process and general undertreatment of PAD risk factors.

There is a wide spectrum of clinical manifestations for PAD: (1) the completely asymptomatic patient found to have PAD from a screening ankle-brachial index (ABI), (2) atypical leg symptoms associated with an exercise limitation, (3) classic intermittent claudication, and (4) ischemic pain and ulceration in the lower extremity from chronic limb ischemia. However, despite the level and degree of limb symptoms, even asymptomatic persons with PAD have a greatly reduced functional capacity. This suggests that occlusive disease in the lower extremity is associated with reduced exercise capacity and functional status regardless of the symptomatic state.

As noted above, symptomatic and asymptomatic PAD is associated with an increased risk for morbidity and mortality, and for impairment of quality of life, as well. A prospective cohort in subjects >65 years of age found a similar high ischemic risk in symptomatic and asymptomatic adults with PAD. Pooled data from 11 studies in 6 countries found that PAD, defined by an ABI of <0.90 was associated with an increased risk of subsequent all-cause mortality (relative risk 1.60), cardiovascular mortality (relative risk 1.96), coronary heart disease (relative risk 1.45), and stroke (relative risk 1.35) after adjustment for age, sex, conventional cardiovascular risk factors, and prevalent cardiovascular disease.

The treatment of PAD has evolved over the past decade to include a broad approach, focusing on the reduction of adverse cardiovascular events, improving symptoms in claudication, and preventing tissue loss in critical limb ischemia. Because quality of life is severely limited in these subjects, great emphasis is placed on ameliorating symptoms and reducing the risk of PAD progression.

Established Therapies for Management of the Systemic Atherothrombosis in PAD

Several sets of guidelines provide physicians taking care of PAD subjects with a list of recommendations regarding optimal treatment recommendations. These include the American College of Cardiology Foundation/American Heart Association guidelines and the international Inter-Society Consensus (TASC) II guidelines. Both are undergoing substantial revision at this time. However, only 15% of the American College of Cardiology Foundation/American Heart Association PAD guidelines come from evidence that is level A. Thus, there is a paucity of high-level data to guide decision making.

Many of the medical therapeutics used in PAD target atherothrombotic pathways. For example, statins, antiplatelets, and angiotensin-converting enzyme inhibitors are used in PAD; yet, much of the data supporting their use stem from data derived from patients with coronary or cerebrovascular disease. For example, the Heart Protection Study (HPS), which evaluated simvastatin versus placebo, and the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial enrolled patients with atherosclerosis in several vascular territories, including a large subgroup of patients with PAD. Both studies found that in general PAD responds similarly to non-PAD (except maybe somewhat better to clopidogrel than to aspirin).

Antiplatelet Therapy

Despite an abundance of data demonstrating the efficacy of antiplatelet therapy in coronary artery disease and cerebrovascular disease, the data in PAD are less compelling. In the Antithrombotic Trialists’ Collaborative meta-analysis, there was a significant 23% lowering of cardiovascular events from a combined analysis of all antiplatelet agents, but the beneficial effects were driven by picotamide (an inhibitor of thromboxane A2 synthase and thromboxane A2 receptors).

Although antiplatelet therapy is effective at decreasing cardiovascular events in patients with PAD, these drugs have no effect on symptomatic improvement in subjects with intermittent claudication.

Aspirin, the most widely used antiplatelet agent in the world, does not have compelling evidence supporting a reduction in cardiovascular events in patients with PAD (despite a positive...
trend). In a recent meta-analysis of 18 prospective randomized trials comprising 5269 subjects with PAD, aspirin resulted in a 12% reduction of the combined end point of nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death that failed to reach statistical significance. Whether a larger sample would provide more convincing benefit is unknown, but recent studies of aspirin in subjects with asymptomatic PAD or PAD with diabetes mellitus were entirely negative. However, the scarcity of randomized data investigating aspirin in symptomatic PAD compared with coronary artery disease or cerebrovascular disease remains a major limitation in clinical decision making.

There is some evidence that clopidogrel monotherapy may be particularly effective in PAD. In a subgroup analysis of CAPRIE, clopidogrel (versus aspirin) had its greatest effect on reducing cardiovascular events in subjects with PAD (P for heterogeneity = 0.045). Subsequently, the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) trial evaluated the effect of aspirin plus clopidogrel versus aspirin alone in patients with established and at risk for cardiovascular disease. In a subgroup analysis of PAD patients, no benefit was observed for dual-antiplatelet therapy with aspirin plus clopidogrel.

Current guidelines recommend antiplatelet therapy in patients with PAD. Because the majority of patients with PAD have concomitant symptomatic coronary artery disease or cerebrovascular disease, aspirin is an acceptable antiplatelet agent in this setting. In addition to aspirin, clopidogrel monotherapy is a reasonable alternative strategy. Implicit in these recommendations is that a large prospective randomized trial investigating the optimal antiplatelet strategy in patients with PAD is long overdue.

Established Therapies for Symptomatic Improvement

Over the past decade there has been a tremendous growth of PAD limb-specific clinical studies suggesting that treatment with metabolic agents, antimicrobials, cell therapy, and gene therapy would result in improved lower-extremity function and viability in subjects with PAD. Trials in humans have led to inconsistent results. This review will provide the current state of medical therapy in subjects with PAD (Table 1). This will include the evidence for and against these new modalities with a focus on medical treatments including drugs and biological agents. Revascularization trials are not within the scope of this review.

Exercise Training

Exercise rehabilitation is a class I, level of evidence A, recommendation for the treatment of claudication in patients with PAD. A landmark meta-analysis in 1995 demonstrated that supervised exercise improves claudication symptoms and increases pain-free walking distance on a treadmill by >100%. In 2008, a rigorous systematic review by the Cochrane group involving 1200 participants from 22 randomized trials with stable claudication demonstrated a significant benefit in improving treadmill walking time and walking distance with a supervised exercise program. Although no benefit was seen in reducing major adverse cardiovascular events or on improving the ABI, subjects randomly assigned to exercise had improvement in claudication symptoms out to 2 years.

Although supervised exercise is very effective in PAD subjects with claudication, several questions remain: (1) is exercise effective in PAD subjects without claudication and (2) what is the role of a nonsupervised exercise program? Two recent randomized trials address these issues. The effect of supervised treadmill exercise or lower-extremity resistance training on functional performance and quality of life was evaluated in a randomized trial of 156 PAD patients, of whom <20% had symptoms of classic claudication. Although any exercise (treadmill or resistance training) improved functional performance, the treadmill exercise group had greater increases in the 6-minute walk distance and in the maximum treadmill walking time in comparison with the resistance group. Of note, the changes in the primary outcomes were similar between subjects with and without claudication and between asymptomatic and symptomatic participants. Despite the efficacy of a supervised exercise program in PAD, lack of reimbursement in the United States remains a major barrier to utilization of this treatment.

Evaluating the role of home-based exercise, Gardner and colleagues randomly assigned 119 subjects with claudication to either home-based exercise, supervised exercise, or a usual care control group. Both exercise programs increased claudication onset time and peak treadmill walking time versus the control group; however, the changes in average walking cadence time were greater in the home-based exercise group. Although this study was small, and nearly 25% of subjects did not complete follow-up, it suggests that a quantified home-based approach may be useful in this high-risk population with impaired quality of life. However, a meta-analysis of home-based exercise training in PAD was negative. Future exercise studies with improved home-training methods, larger populations, and clinically meaningful end points are certainly warranted. In the meantime, subjects with PAD (symptomatic or not) are likely to benefit from an aggressive exercise regimen.

Cilostazol

Cilostazol is a phosphodiesterase type 3 inhibitor approved in the United States in 1999 to treat intermittent claudication. Its possible mechanism of action includes increasing intracellu-

### Table 1. Effect of Medical Therapies in Subjects With Peripheral Artery Disease

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Symptomatic Improvement</th>
<th>Reduced Cardiovascular Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>++ +</td>
<td>Not studied</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>++</td>
<td>Neutral</td>
</tr>
<tr>
<td>Statins</td>
<td>+/−</td>
<td>Neutral</td>
</tr>
<tr>
<td>Antiplatelets</td>
<td>−</td>
<td>+++</td>
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<tr>
<td>L-Carnitine and propionyl-L-carnitine</td>
<td>++</td>
<td>Not studied</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>++</td>
<td>Not studied</td>
</tr>
<tr>
<td>Naftidrofuryl</td>
<td>++</td>
<td>Not studied</td>
</tr>
<tr>
<td>Gene therapy</td>
<td>+/−</td>
<td>Neutral</td>
</tr>
<tr>
<td>Cell therapy</td>
<td>+/−</td>
<td>Not studied</td>
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</table>
lar concentrations of cAMP, thereby causing vasodilation and inhibiting platelet aggregation. Although other drugs with vasodilating and platelet-inhibiting properties have not been demonstrated to improve claudication symptoms, cilostazol is effective in ameliorating symptoms. A meta-analysis of 8 randomized trials including 2702 PAD subjects with claudication found that cilostazol improved maximum and pain-free treadmill walking distance and quality-of-life measures. Of note, cilostazol decreased triglyceride concentration by 16% and increased high-density lipoprotein levels by 13%, but also increased the incidence of headache, bowel concerns, and palpitations.

Cilostazol may have an additional benefit of reducing restenosis and repeat revascularization following endovascular therapy. Similar to patients with coronary artery disease, patients with PAD have lower rates of target vessel revascularization following cilostazol therapy. However, these studies have been small and open label in design, thus hypothesis generating.

Milrinone, another phosphodiesterase type 3 inhibitor, was noted to increase mortality in subjects with heart failure. In response, the US Food and Drug Administration mandated a long-term safety study of cilostazol. Hiatt and colleagues performed a phase 4 (postmarketing) randomized, double-blind, placebo-controlled trial of cilostazol in 1435 PAD subjects with claudication. Although underpowered because of a lower mortality than projected and poor study drug adherence, this is the largest study to date that has evaluated serious adverse events and mortality. No difference was observed for overall mortality or serious bleeding events between the cilostazol and placebo groups; however, the study could not exclude a modest increased risk. In comparison with milrinone, cilostazol has fewer cardiac inotropic effects but equivalent vasodilating and platelet-inhibiting properties. Nonetheless, an advisory from the US Food and Drug Administration stated that cilostazol should not be used in subjects with congestive heart failure.

**Statin Therapy**

Subgroup analyses of PAD patients from large randomized trial data in subjects with PAD found a reduction in cardiovascular events from statin therapy. Exploratory analyses suggested an improvement of claudication symptoms in subjects with PAD. In a randomized trial of 354 PAD subjects with claudication, Mohler and colleagues demonstrated that atorvastatin (10 or 80 mg daily) improved pain-free walking distance and community-based physical activity. However, the functional benefits of statins on claudication have not been proven.

**Carnitine and Propionyl-L-Carnitine Therapy**

Carnitine and propionyl-L-carnitine may improve muscle metabolism and exercise performance in patients with PAD. Initial studies evaluated carnitine given orally as 2 g twice daily, and also given as a single 3-g dose intravenously. These early studies in PAD were indicative of clinical benefit and therefore led to the development of propionyl-L-carnitine, an acyl form of L-carnitine.

In 2 published phase 3 trials, propionyl-L-carnitine had a significant improvement on claudication-limited treadmill exercise performance. In Europe, a total of 501 subjects were randomly assigned to propionyl-L-carnitine 2 g/d for 12 months (n=248) or placebo (n=253). The primary analysis was the subgroup of 171 patients who had a maximum walking distance between 50 and 250 m and baseline variability ≤25%. After 12 months of treatment, patients randomly assigned to placebo increase maximal walking distance 44% versus 61% on drug. This difference had a probability value of 0.055 by the primary analysis and 0.048 with a secondary analysis. The quality-of-life questionnaire was also positive in favor of drug (P=0.002). In the second pivotal trial, a total of 161 subjects were randomly assigned to propionyl-L-carnitine, 2 g/d (n=85), or placebo (n=76). After 6 months of therapy, treated patients increased peak walking time 78% with a 37% increase in controls (P=0.002). Patients randomly assigned to propionyl-L-carnitine also had significant improvements in the physical function scores of the SF-36 (P=0.31), but not with the Waling Impairment Questionnaire (P=0.26). However, more recent studies in which propionyl-L-carnitine was given on a background of exercise training, although showing favorable trends, were not statistically significant on the primary end point. Thus, the overall incremental benefit of propionyl-L-carnitine in PAD remains to be determined.

**Other Medical Therapies**

### Pentoxifylline

Pentoxifylline is a xanthine derivative used to treat patients with intermittent claudication. Its mechanism of action is thought to be a rheological modifier that includes improving the deformability of red blood cells and white blood cells, and decreasing fibrinogen concentration, platelet adhesiveness, and whole-blood viscosity. A meta-analysis demonstrated a modestly improved walking distance, substantially less effective than either cilostazol or a supervised exercise program.

### Naftidrofuryl

Naftidrofuryl is a serotonin 5-hydroxytryptamine 2 receptor antagonist with vasoactive properties in addition to its effect on oxidative metabolism and rheological properties on the red blood cell and platelet. Although not approved in the United States, it is currently used in Europe for the treatment of claudication. In a patient-level meta-analysis, naftidrofuryl improved symptomatic claudication without any serious adverse events.

**Medical Treatment of the Patient Undergoing Revascularization**

Antiplatelet therapy has good evidence for the prevention of graft occlusion after peripheral vascular surgical procedures. In the Antithrombotic Trialists’ Collaboration meta-analysis, 3000 patients with peripheral artery procedures had a 16% graft occlusion rate on antiplatelet therapy in comparison with 25% in the control group (P<0.00001). Aspirin with or without other antiplatelet therapy was associated with a significantly lower risk of graft occlusion. Ticlopidine has also been shown to promote vein graft patency without any difference in cardiovascular events. Dual-antiplatelet therapy with aspirin plus clopidogrel versus aspirin alone was tested in the clopidogrel and acetylsalicylic acid in bypass surgery for peripheral arterial disease (CASPAR) trial.
There was no significant difference in the primary end point (graft occlusion, amputation, or death) between groups. In a subgroup analysis, clopidogrel plus aspirin was better than aspirin alone among patients who received prosthetic grafts. As expected, bleeding was more common in the dual-antiplatelet therapy group.47

Anticoagulation has also been recommended as an adjuvant to maintain surgical graft patency.47 The use of heparin anticoagulation in the immediate postoperative period, particularly low-molecular-weight heparin, may improve the patency of infrainguinal bypass grafts (vein and prosthetic), but has also been associated with increased bleeding.48,49 The role of long-term oral anticoagulation is more controversial. In one trial of patients undergoing infrainguinal bypass surgery (including the use of vein, prosthetic material, and endarterectomy), long-term warfarin was associated with an increased bleeding risk but no benefit in patency or survival.50 In contrast, another study performed in a similar population demonstrated that oral anticoagulation improved graft patency, limb salvage, and survival.51

Several trials have compared the benefits of anticoagulation versus antiplatelet therapy. Among patients treated with infrainguinal bypass procedures (predominantly using prosthetic material), randomization to low-molecular-weight heparin versus aspirin and dipyridamole was associated with better graft patency, especially in the patients treated for critical limb ischemia.52 The Dutch Bypass Oral Anticoagulants or Aspirin study evaluated 2690 patients undergoing infrainguinal bypass.53 Half the patients were treated for claudication and the other half were treated for critical limb ischemia with a fairly even distribution of vein versus prosthetic material. The aspirin dose was 80 mg/d, and, in patients randomly assigned to anticoagulation, the international normalized ratio was maintained at 3.0 to 4.5. The primary end point of patency was equal between groups after 21 months follow-up. In subgroup analysis, anticoagulation maintained vein graft patency better than aspirin but at a higher risk of bleeding complications. In contrast, aspirin maintained prosthetic graft patency better than anticoagulation. Although these results suggest that patients receiving vein grafts should be preferentially treated with warfarin and those with prosthetic material with aspirin, adequately powered studies are needed to determine the optimal antithrombotic and antiplatelet strategy following vascular surgery.

In the context of promoting patency after a revascularization procedure, antiplatelet and antiangiogenic therapy have a clear role, but the data are somewhat dated. In terms of selection between type of therapy, aspirin may be favored for prosthetic grafts and anticoagulation for vein grafts or for higher-risk patients for occlusion.44 Future trials are needed.

**Novel Therapies**

Therapeutic angiogenesis is a promising investigational strategy for the treatment of patients with claudication and chronic leg ischemia (CLI). It is an application of biotechnology to stimulate new vessel formation via local administration of proangiogenic growth factors delivered as recombinant protein or by gene therapy, or by implantation of endothelial or other progenitor cells that will synthesize multiple angiogenic cytokines.

**Gene Therapy**

Gene therapy involves transfer of genetic material into cells to modify their genetic expression to produce a clinically useful effect on angiogenesis, capillary generation, and collateral formation. Clinical trials have sought to establish the role for therapeutic angiogenesis by use of gene transfer with proangiogenic factors mediated by plasmids or viral vectors in patients with PAD, although results have been inconsistent (Table 2). These proangiogenic factors include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hypoxia-inducible factor (HIF)-1α, and hepatocyte growth factor (HGF).

In a meta-analysis evaluating the efficacy and safety of different gene therapies, recombinant protein and cellular-based treatment approaches in patients with PAD, therapeutic angiogenesis was associated with a modest, albeit significant clinical improvement in peak walking time, ulcer healing, rest pain relief, and limb salvage versus placebo in subjects with PAD (odds ratio 1.44, 95% CI 1.03–2.00).62 The improvement was most impressive in subjects with CLI. There was also a 30% increase in the odds of the adverse events of edema, hypotension, and proteinuria. No significant difference was detected for the endpoints of mortality, malignancy, or retinopathy.

**Vascular Endothelial Growth Factor**

Vascular endothelial growth factor is expressed under hypoxic conditions and is a potent regulator of endothelial cell migration, proliferation, repair, and survival.63 In previous studies involving animal studies, VEGF gene transfer induced rapid production of nitric oxide and prostacyclin from endothelium causing a vasculoprotective effect.64–65 In a hindlimb ischemia animal model, VEGF was demonstrated to induce angiogenesis and arteriogenesis.66–67 Initial uncontrolled clinical trials have suggested promising therapeutic effects with naked VEGF plasmid gene transfer.68,69 In a comparison of intra-arterial infusion of adenoviral VEGF165, plasmid liposome VEGF165, or Ringers lactate placebo, Makinen et al70 noted that both VEGF165 treatments appeared safe and were associated with significant increases in vascularity.

In a phase I trial, Baumgartner and colleagues70 found that intramuscular injection achieves overexpression of VEGF sufficient to induce therapeutic angiogenesis in selected patients with CLI. In the Regional Angiogenesis with VEGF (RAVE) trial, a single intramuscular adenoviral delivery of VEGF121 in patients with unilateral exercise-limiting claudication failed to achieve its primary end point of change in treadmill peak walking time.54 There was no difference in any of the secondary end points, and VEGF121 was associated with a dose-dependent increase in peripheral edema. Reasons for the lack of efficacy could be the population selected (patients with bilateral PAD were excluded), the duration of expression of the VEGF121 transgene by use of the adenoviral approach may be insufficient to induce a phenotypic response, and the single injection may be insufficient.71 It is possible that
locally expressed VEGF$_{121}$ has a short tissue half-life and, consequently, induces only the first step in angiogenesis, whereas the longer tissue retention of VEGF$_{165}$ may permit execution of the full paradigm of angiogenesis.

In a small randomized trial, Kusumanto et al.$^{55}$ evaluated the effect of 2 intramuscular injections of plasmid-encoded VEGF$_{165}$ versus placebo in 54 diabetic patients with PAD and CLI. The authors claim the study was powered to detect a 25% absolute difference in major amputations. There were numerically fewer major amputations (3 [11%] versus 6 [22%]), but this difference failed to reach statistical significance. There was more skin ulcer healing and increased hemodynamic improvement in the VEGF$_{165}$ group.

Fibroblast Growth Factor

Fibroblast growth factor modulates and enhances new blood vessel formation and activates migration, proliferation, and differentiation of endothelial cells, resulting in angiogenesis. FGF acts on endothelial cells, smooth muscle cells, and fibroblasts via an interaction with specific receptors on the cell surface. There are 22 known FGF ligands that are involved in angiogenesis.$^{71}$ FGF-1 and FGF-2 have been studied in human PAD gene therapy trials. FGF-1 and FGF-2 are different from other FGF proteins in lacking a signal sequence for extracellular transport.$^{72}$ In a phase I trial, intramuscular administration of nonviral 1 fibroblast growth factor (NV1FGF) to limbs of patients with CLI was well tolerated.$^{73}$ In an open-label design, patients experienced improvements in wound healing, pain, transcutaneous partial pressure of oxygen, and ABI. The Therapeutic Angiogenesis with Intramuscular NV1FGF Improves Amputation-Free Survival in Patients with Critical Limb Ischemia (TALISMAN 201) trial was a phase II trial in patients with CLI.$^{58}$ There was no difference in the primary end point of ulcer healing between the NV1FGF group and placebo group. However, use of NV1FGF significantly reduced the secondary end point of risk of all amputations by $50\%$ with a nonsignificant trend toward a lower mortality. These results served as the basis for a large phase 3 trial of NV1FGF on amputation-free survival.

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Table 2. Phase II and Phase III Randomized, Controlled Trials of Gene Therapy and Cell Therapy in Subjects With Peripheral Artery Disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Population</th>
<th>No.</th>
<th>Treatment</th>
<th>Follow-Up</th>
<th>Primary End Point</th>
<th>Findings</th>
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<td></td>
<td></td>
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<tr>
<td>RAVE$^{54}$</td>
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<td>Claudication</td>
<td>105</td>
<td>VEGF$_{121}$</td>
<td>12 wk</td>
<td>Peak walking time</td>
<td>No benefit. Dose-dependent peripheral edema</td>
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<tr>
<td>Kusumanto et al.$^{55}$</td>
<td>II</td>
<td>Diabetics with CLI</td>
<td>54</td>
<td>VEGF$_{165}$</td>
<td>100 d</td>
<td>Major amputation</td>
<td>No benefit in primary EP, but improvement in hemodynamics and skin ulcer healing</td>
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<tr>
<td>DELTA$^{56}$</td>
<td>II</td>
<td>Claudication</td>
<td>105</td>
<td>Del-1</td>
<td>90 d</td>
<td>Peak walking time</td>
<td>No benefit and no major safety issues</td>
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<td>TRAFFIC$^{57}$</td>
<td>II</td>
<td>Claudication</td>
<td>190</td>
<td>FGF-2 protein</td>
<td>90 d</td>
<td>Peak walking time</td>
<td>Significant benefit in primary EP. No major safety concern</td>
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<td>TALISMAN$^{58}$</td>
<td>II</td>
<td>CLI</td>
<td>125</td>
<td>NV1FGF</td>
<td>25 wk</td>
<td>Ulcer healing</td>
<td>No benefit in the primary EP. Secondary of amputation and death was reduced. No major safety issues</td>
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<tr>
<td>TAMARIS$^{59}$</td>
<td>III</td>
<td>CLI</td>
<td>525</td>
<td>NV1FGF</td>
<td>1 y</td>
<td>Death or major amputation</td>
<td>No benefit and no major safety issues (malignancy, retinopathy, or proteinuria)</td>
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<td>Cell therapy</td>
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<tr>
<td>START$^{60}$</td>
<td>II</td>
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<td>40</td>
<td>GM-CSF</td>
<td>14 d</td>
<td>Walking distance</td>
<td>No benefit</td>
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<tr>
<td>Arai et al.$^{61}$</td>
<td>II</td>
<td>CLI</td>
<td>39</td>
<td>G-CSF</td>
<td>1 mo</td>
<td>Safety and feasibility</td>
<td>No significant adverse events. Improvement in ABI and transcutaneous oxygen pressure</td>
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</table>

CLl indicates critical leg ischemia; VEGF, vascular endothelial growth factor; Del-1, Developmentally Regulated Endothelial Locus; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; NV1FGF, nonviral 1 fibroblast growth factor; ABI, ankle-brachial index; and EP, end point.
importance of performing large phase III trials in this new area of gene therapeutics.

In the setting of claudication, Lederman and colleagues performed the Therapeutic Angiogenesis with FGF-2 for Intermittent Claudication (TRAFFIC) phase II trial testing the efficacy and safety of intra-arterial FGF-2 protein in 190 patients with moderate-to-severe intermittent claudication. Intra-arterial FGF-2 protein resulted in a significant increase in peak walking time at 90 days. No follow-up study has been performed.

Hypoxia Inducible Factor-1α
The transcription factor HIF-1α is important in vascular hemostasis; HIF-1α regulates the expression of specific genes involved in the response to hypoxia and wound healing. Because HIF-1α involves both physiological and pathological angiogenesis, strategies that target this factor have been tested in the setting of PAD. In a phase I dose escalation study, HIF-1α delivered intramuscularly with an adenoviral vector was safe out to 1-year. There were data to suggest that certain subjects had amelioration of rest pain symptoms and complete ulcer healing. In a larger study (WALK) to assess the transcription factor HIF-1 α, results demonstrated no benefit in the primary end point.

Hepatocyte Growth Factor
Hepatocyte growth factor stimulates the growth of endothelial cells and promotes angiogenesis. HGF is able to induce robust collateral formation in preliminary studies. Subsequently, a randomized trial was performed to assess the safety and potential efficacy of intramuscular injections of HGF plasmids in 104 patients with CLI. There was no safety concern attributed to the HGF therapy. Seventy-three patients were available for the efficacy component; the highest-dose–treated group had a significant increase in transcutaneous partial pressure of oxygen at 6 months, which was significantly greater than in the placebo group. There was no difference between groups in the ABI, toe-brachial index, rest pain, and ulcer healing. In a nonrandomized comparison, patients had an improvement in ABI, toe-brachial index, pain relief, wound healing, or major amputation. In a recent phase II/IIIa study, Morishita and colleagues evaluated the safety and potential efficacy of intramuscular injection of plasmid HGF in patients with CLI. No serious adverse events were noted. In a nonrandomized comparison, patients had an improvement in ABI, toe-brachial index, rest pain, and ulcer size at 6 months. There was no difference in transcutaneous partial pressure of oxygen. Larger studies are needed to determine the efficacy and safety profile of HGF.

Developmentally Regulated Endothelial Locus
Developmentally Regulated Endothelial Locus (Del-1) is an extracellular matrix protein that accumulates around angiogenic blood vessels and promotes angiogenesis even in the absence of exogenous growth factors. The Del-1 for Therapeutic Angiogenesis (DELTA) trial randomly assigned 105 PAD patients with claudication to intramuscular injections of Del-1 plasmid with poloxamer 188 (to enhance transfection) or poloxamer 188 alone. No significant safety issues were associated with Del-1. Peak treadmill walking time improved significantly in both groups without any incremental benefit in the Del-1 group. Although ABI, claudication onset time, and quality of life improved in both groups, no difference emerged between Del-1 and the control group. The improvement of outcomes in both groups observed in this study underscores the substantial placebo effect and the importance of having a placebo group.

Advancement of gene therapy from the safety-and-feasibility stage to routine clinical use will require carefully designed, adequately powered, large-scale, randomized, controlled phase III trials that incorporate end points that address methodological improvements, long-term safety, and clinically important end points.

Cell Therapy
Another potential novel treatment modality for PAD patients with claudication and chronic ischemia is the stimulation of angiogenesis with cell therapy. Endothelial progenitor cells are important mediators in postnatal neovascularization after mobilization from the bone marrow. Numerous studies have demonstrated that, in the setting of both hindlimb and coronary ischemia models, endothelial progenitor cells can be harvested, expanded ex vivo, and delivered to augment capillary density, perfusion, and organ function. Bone marrow–derived mononuclear cell implantation stimulates the production of endothelial progenitor cells and increases collateral vessel formation in both ischemic limb models and patients with limb ischemia. Implantation of bone marrow mononuclear cells (versus peripheral blood mononuclear cells) improved the ABI, transcutaneous oxygen pressure, rest pain, and pain-free treadmill walking time at 4 and 24 weeks after injection. In a follow-up study, Higashi and colleagues demonstrated that bone marrow mononuclear cells improved endothelium-dependent vasodilation in patients with limb ischemia in addition to an effect on ischemic symptoms and findings of angiography. Several international phase II trials are underway to assess the benefit/safety profile of cell therapy in patients with PAD.

In an animal model, intra-arterial infusion of granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the development of arterial collateral blood vessels following femoral artery occlusion. In a small study of 21 subjects with extensive coronary artery disease not eligible for bypass surgery, intracoronary injection of GM-CSF improved collateral flow and led to a reduction in ST-segment changes and episodes of angina. The STimulation of ARTeriogenesis (START) study was the first placebo-controlled study in the setting of moderate or severe claudication to investigate the effect of GM-CSF on walking distance. Patients were treated with placebo or subcutaneously applied GM-CSF (10 μg/kg) for a period of 14 days. No major side effects were observed; however, skin rash, muscle pain, and fever were more common in the GM-CSF group. There was no difference in the primary end point (increase in maximum walking distance) or secondary end point (ABI) either directly after treatment or at 90 days of follow-up. Treatment with granulocyte colony-stimulating factor is another promising approach in this setting.
Antimicrobials

Observational data provided the groundwork suggesting that an infectious agent may be an important link between chronic inflammation and PAD. In a small study, 20 subjects seropositive to \textit{Chlamydia} pneumoniae who received roxithromycin (400 mg daily) had better walking distance and fewer invasive revascularization procedures than 31 subjects seropositive for \textit{Chlamydia} pneumoniae who did not receive antibiotics.\textsuperscript{89} Several other small studies failed to demonstrate a benefit of antimicrobial therapy in subjects with PAD.\textsuperscript{90,91} To better understand the role of antimicrobials in PAD, Jaff and colleagues\textsuperscript{92} randomly assigned 283 PAD subjects with claudication to 25 mg rifalazil weekly for 8 weeks or matching placebo. All subjects enrolled had \textit{Chlamydia} pneumoniae antibody titer values \textgreater{} 1:128. There was no benefit of rifalazil on the primary or any secondary assessment measured.\textsuperscript{82} No difference in cardiovascular events was noted, but the study was underpowered to detect this difference. Combined with the plethora of negative studies of antimicrobials in subjects with coronary disease,\textsuperscript{93} there is little compelling reason to study this class of compounds in subjects with atherosclerotic disease.

Future Directions

Medical management of PAD has gone through important innovations over the past several decades with the widespread use of exercise, antiplatelet therapy, and statin medications. However, many of the recommendations for patients with PAD were derived from subgroup analyses of trials performed in populations with other atherosclerotic disease manifestations. Even aspirin, a class I level of recommendation A therapy used in the treatment of PAD, has not been demonstrated to decrease cardiovascular events in the setting of PAD. There are considerable gaps in knowledge regarding the optimal treatment of PAD patients with claudication and CLI in comparison with patients with atherosclerotic disease in other territories. Current need for improvement in PAD patient outcomes include (1) PAD-focused systemic therapies designed to reduce the risk of ischemic events in the target population, and (2) continued development of limb-specific therapies, particularly in CLI.

Conclusions

Despite the paucity of PAD-specific limb and systemic therapies, PAD patients are at very high risk for cardiovascular events and impaired quality of life and should therefore be treated aggressively. Until additional prospective, randomized trials are performed in this population, patients should be treated with strategies based on current recommendations for other manifestations of atherosclerotic disease. Current pharmacological options include cilostazol for claudication but little else to recommend as other claudication therapies or medical approaches to CLI.

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