Peripheral arterial disease (PAD) is a common disorder and a major cause of morbidity and mortality. The most severely affected patients, with rest pain, ulcerations, or gangrene, are given a diagnosis of critical limb ischemia (CLI). These patients have a particularly poor prognosis, with high rates of limb amputation and mortality. Despite improvements in medical therapy for atherosclerosis and associated comorbidities as well as improvements in interventional and surgical techniques to improve limb perfusion, CLI continues to carry a major risk of limb amputation. A significant portion of patients with CLI are considered “no option” for revascularization, and no medical therapy has been shown to be capable of reducing the need for amputation. Therefore, novel therapies are needed to treat this disorder.

The therapeutic use of progenitor cells poses certain unique questions in clinical trial design. For example, in addition to the usual experimental variables that would be considered for a pharmaceutical agent, such as the dose of the drug, the patient population, and the end points, cardiovascular cell therapy studies must consider many additional variables, including the source of stem/progenitor cells, the method of obtaining cells, cell processing protocols, the selection of cell subtypes, the route of cell delivery, and whether a single dose or multiple dose regimen will be tested. In addition, as a biological therapy, extensive quality control measures must be in place to ensure safety and to evaluate the impact of cell phenotype on efficacy. In this issue of *Circulation: Cardiovascular Interventions*, 2 studies evaluate the efficacy of bone marrow mononuclear cell (BM-MNC) therapy for subjects with CLI, and both simultaneously advance our understanding and underscore the challenges in this field.

Idei and colleagues report the results of their experience using intramuscular injection of BM-MNCs for the treatment of CLI. They studied 51 subjects treated with BM-MNCs and 46 subjects with CLI with similar baseline demographics who were not treated with BM-MNCs. This study was not randomized, placebo controlled, or blinded. Although the control group had similar characteristics to the BM-MNC-treated subjects, the reported differences in amputation-free survival are striking. One of the most notable aspects of the findings is the extremely high incidence of amputation among the control subjects, with 0% amputation-free survival in patients with PAD and 10% amputation-free survival in patients with Buerger disease. Even allowing for the small sample size, these numbers are difficult to reconcile with the existing literature.

For example, Ohta et al performed a retrospective study of 110 patients with Buerger disease with a mean follow-up of 10.6 years and found a cumulative 43.6% combined minor and major amputation rate, whereas Cooper et al performed a retrospective study of 111 patients with Buerger disease with a mean follow-up of 15.6 years and found a major amputation rate of 11% at 5 years and a total amputation rate of 25% at 5 years. Recent clinical trials in subjects with CLI with atherosclerotic PAD revealed total amputation rates in the range of 50% to 60% at 1 year.

Thus, one must consider the possibility of a selection bias when comparing outcomes in this nonrandomized clinical trial. Indeed, the Methods section states that treated subjects were enrolled if they had rest pain or nonhealing ulcers, whereas control subjects were included on being considered for major amputation. It is also noted that smoking cessation was required before administration for BM-MNC but not so for the control group. It is therefore not clear how a statistical comparison of these apparently different populations can be meaningful. A comparison of treated patients with historical controls might have been informative, although recent experience in CLI studies has underscored the need for contem-
poraneous controls in a randomized, blinded design. For example, in a phase II randomized controlled trial of FGF1 gene therapy in 107 patients with CLI, the 1-year major amputation rate was 34% but was only 21% in the phase III study of the same agent in 525 patients. The approach in the study by Idea et al replicates the Therapeutic Angiogenesis using Cell Transplantation (TACT) study; however, the present investigation provides certain advances compared to that initial landmark study, for example, much longer follow-up of the patients. In this regard, it is interesting to note that the physiological end points of ankle-brachial index (ABI) and transcutaneous oximetry parallel those shown in TACT up to 6 months, which was the final end point shown in that study. Longer-term follow-up by Idei et al reveals persistent improvement in these parameters in the patients with Buerger disease, whereas those with atherosclerotic PAD had worsening of these parameters at later time points. This finding may have important implications for the role of readministration strategies, as is further illustrated in the study by Walter et al, which is discussed later.

In an interesting in vitro study, Idei et al provide evidence that autologous cells from subjects with Buerger disease have preserved phenotypic characteristics, whereas autologous cells from subjects with atherosclerotic PAD are impaired. In addition, the number of endothelial progenitor cells was lower in subjects with PAD versus those with Buerger disease. Patients with Buerger disease generally have a low burden of atherosclerotic risk factors because, by definition, they have onset of disease before age 50 and absence of typical atherosclerotic risk factors other than smoking history. Because it is now well established that risk factors for atherosclerosis impair the function of autologous stem/progenitor cells, it is interesting to contemplate the possibility that the greater response among subjects with Buerger disease could be partly due to higher-functioning stem/progenitor cells in these patients. Therefore, the observations of Idei and colleagues underscore the importance of cell number and function as potentially critical determinants of the clinical efficacy of autologous cell therapy.

In the second study, Walter and colleagues report the results of the randomized, double-blind, placebo-controlled PROVASA (Intraarterial Progenitor Cell Transplantation of Bone Marrow Mononuclear Cells for Induction of Neovascularization in Patients With Peripheral Arterial Occlusive Disease) study. In this study, 40 patients were randomized in a 1:1 fashion to intraarterial delivery of either BM-MNCs or placebo. The PROVASA study used an innovative randomized-start clinical trial design. After initial randomization and treatment with either BM-MNCs or placebo, patients were followed for 3 months. At this point, placebo-treated patients crossed over to active treatment and active-treated patients received a second treatment of BM-MNCs. This unique protocol allowed the PROVASA investigators to determine whether repeated treatments of autologous cell therapy may be beneficial compared with a single treatment. This is important because randomized trials of cardiovascular cell therapy to date have primarily used a single administration of cells, and as shown in the follow-up data from Idei et al, there is evidence that a single administration may have a limited duration of effect.

The PROVASA investigators found no significant difference in the primary outcome of improvement in ABI. However, there were significant improvements in important and highly clinically relevant secondary end points, including ulcer healing and rest pain reduction in subjects treated with BM-MNCs versus placebo. In addition, a number of important observations were made. First, patients with Rutherford class 6 CLI (gangrene or major tissue loss) at baseline did not respond to therapy. All these patients had poor outcomes, most likely owing to the fact that the disease process is too far advanced and the clinical outcome all but predetermined in these unfortunate individuals. Second, the investigators identified that major predictors of successful ulcer healing included total cell number delivered, repeated cell administration, and greater cell functionality measured by in vitro assays. This study provides further evidence that cell function is an important predictor of response to therapy. In addition, a higher number of total BM-MNCs and CD34+ cells delivered correlated with superior outcomes. Perhaps most importantly, the PROVASA investigators demonstrated that multiple treatments of BM-MNCs were associated with significantly greater improvements in ulcer healing and rest pain than a single treatment. This finding provides the rationale for future trials of cardiovascular cell therapy to include multiple treatments in their study design.

Why did the PROVASA study fail to meet its primary end point of change in ABI? The authors believe that change in ABI was a poor selection as a primary end point because they did not find a correlation between change in ABI and improvement in ulcer healing or improvement in rest pain. This same divergence between surrogate end points like ABI or transcutaneous oximetry and hard clinical end points has been noted in other CLI studies and remains a major challenge in designing phase II studies for this condition. Looking at Figure 2 in Walter et al, one can tell that there is significant variation within each group in the ABI measurement, even at baseline. The authors correctly point out that the best efficacy end point for clinical trials in the CLI population is still uncertain and that a composite end point may be most appropriate. Amputation-free survival is a good hard end point for CLI studies; however, this study was not designed or powered to demonstrate a difference in this end point, especially because the randomized-start design allowed the placebo-treated patients to cross over to active treatment after 3 months.

The choice of change in ABI as the primary end point and the power calculations for this study were based on the TACT study, which demonstrated significant improvements in ABI as well as transcutaneous oxygen pressure, rest pain, and pain-free walking time with BM-MNCs. The TACT study, however, did not have a conventional control group; 1 arm of the study enrolled patients with bilateral limb ischemia who served as their own controls, with 1 limb treated with BM-MNCs and the other with peripheral blood MNCs as a control. Beyond this difference, there are 2 important differences between the TACT and Idei et al study and the PROVASA study protocols. First, the TACT and Idei et al
studies used intramuscular injection, and PROVASA used intraarterial injection. The effect of this difference is difficult to determine because no studies of human cell therapy for CLI have compared different delivery strategies. Second, the TACT and Idei et al protocols involved a large bone marrow aspiration of ~500 mL and an injection of ~1.6 to 1.8 x 10⁶ BM-MNCs and 3.5 to 3.9 x 10⁶ CD34+ cells, whereas the PROVASA protocol involved a 50-mL bone marrow aspirate and delivery of 1.53 to 1.65 x 10⁶ BM-MNCs and 2.7 to 2.9 x 10⁶ CD34+ cells during each treatment. The TACT protocol had a 10-fold greater volume of bone marrow aspirate and ~10-fold greater total cell delivery and CD34+ cell delivery. Choosing the optimal dose in cell therapy studies is a difficult task because traditional pharmaceutical properties of absorption, distribution, metabolism, and excretion are not easily measured or not applicable. Dosing decisions currently are made by extrapolation from animal models and empirically from efficacy signals in phase I and II clinical trials that compare multiple doses. The challenge of dosing is further complicated by the nonlinear dose-response relationship to manipulations of angiogenesis that have been documented in a variety of settings. Finding the optimal cell dosing strategy will remain a critical issue for the ongoing clinical translation of cardiovascular cell therapy.

Overall, these 2 studies add to our growing knowledge about the clinical translation of cardiovascular cell therapy. They highlight opportunities for cell therapy and emphasize ongoing challenges. Both studies yield insights regarding the potential importance of progenitor cell phenotype and function and therefore will trigger consideration of methods to enhance cell potency before administration. The PROVASA study design addresses an important question of single versus multiple treatments, and future studies likely will include repeated cell treatments in their main protocols or as an option for patients with inadequate response after a single treatment. Addressing the critical issues of dose optimization and improving the function of autologous cells from patients with multiple comorbidities will be major themes as we work to maximize safety and efficacy and position cell therapy to move forward to help patients with currently untreatable conditions.

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