Critical Limb Ischemia and Stem Cell Research
Anchoring Hope With Informed Adverse Event Reporting

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Critical limb ischemia (CLI) represents a syndrome that is associated with a particularly adverse natural history. Although clinicians increasingly recognize that peripheral arterial disease (PAD) includes a broad range of clinical syndromes, CLI is associated with very adverse short-term limb and systemic cardiovascular outcomes.1 CLI is not a specific disease per se; rather, it represents a syndrome that may develop from many fundamentally distinct pathophysiological processes, including advanced atherosclerosis, thromboembolism or atheroembolism, in situ thrombosis, and the arteritides, such as thromboangiitis obliterans (TAO, or Buerger disease).

The Challenge of Thromboangiitis Obliterans

TAO is a form of PAD that is obliterative, thrombotic, and commonly progressive, especially in individuals who sustain exposure to tobacco products.2 Refractory ischemic leg pain, skin ulceration, and gangrene may ensue, with a high short-term risk of amputation. TAO is distinct from other diseases that can obliterate distal arterial beds. It is not adequate to offer a TAO diagnosis to individuals with distal arterial disease who are male and who smoke. Clinicians in practice and investigators should be advised to apply the diagnostic label carefully, such as via use of the Olin criteria.2

For most individuals with CLI attributable to atherosclerosis, the immediate therapeutic goal is reestablishment of limb perfusion via endovascular or surgical methods, with aggressive treatment of the causative risk factors. For individuals with TAO, distal arterial obstruction may obviate successful revascularization, and patency may not be easily maintained. Thus, beyond tobacco cessation, there are few therapeutic options that are evidence based and that can sustain real hope for either short- or long-term improvement. Despite past clinical investigation of potential pharmacotherapies, including prostaglandin analogues, vasodilator medications, and other interventions in limited clinical trials, outcomes remain poor.2,3,4 Because this form of PAD is, by definition, associated with distal arterial obstruction with an associated inflammatory response, neither endovascular nor operative arterial bypass is usually possible, and these interventions are known to be less successful and less durable for this condition.2,5

Medical Therapy for CLI: A Vascular “Orphan Disease”

It should be noted that the TAO could be viewed as a model peripheral arterial orphan disease, as defined by the Food and Drug Administration.6 The true prevalence of TAO is unknown, but incident cases are relatively uncommon. Any new medication or intervention would likely be used by fewer than 200 000 people in the United States. As such, there are particular challenges associated with successful drug development, because there would be no reasonable expectation that the costs of research and development of new pharmacological or cell-based interventions for this indication alone would be recovered by sales of the drug in the United States. In fact, drug development for TAO or any CLI syndrome has been minimal. The incentive to identify pharmacological interventions is now largely sustained by the clinical investigative drive of vascular clinician–scientists who suspect that therapeutic success for any form of CLI (including TAO) would clarify new mechanisms that would be applicable for individuals with more common manifestations of PAD.

PAD has long served as an arena of central interest for investigators seeking to demonstrate that angiogenesis can truly be therapeutic and low risk. Early evidence has defined therapeutic benefit with negligible risk for individuals with PAD and claudication,7 as pain-free walking has improved with administration of recombinant fibroblast growth factor-2. Individuals with PAD and CLI have been evaluated in small studies to demonstrate alleviation of ischemic pain and wound healing.8–11 Such studies have not yet been completed to definitively demonstrate diminished rates of amputation or improvements in amputation-free survival.

Most recently, it has been suggested that angiogenesis could be induced by administration of endothelial progenitor cells, which are known to be preferentially recruited to sites of injury at which they might repair damaged tissues.12 Preclinical data have shown that bone marrow–derived mononuclear cells (BM-MNCs), which include endothelial progenitor cells, have a predilection for the ischemic hind limb where angiogenesis can be induced.13 Thus, for both patients and clinicians who provide care for individuals with CLI, therapeutic angiogenesis by use of genes, proteins, or cell therapies has provided a conceptual avenue that has continued to offer hope.14
The Current Data: Limited, but Meriting Interpretation

The article by Miyamoto et al13 published in this issue of *Circulation* provides data from a very limited series of patients treated with BM-MNCs and provides both hope and caution. The authors administered autologous BM-MNC with the intent of inducing an angiogenic response, with observable therapeutic benefit and acceptable risk. These data demonstrate (from a particularly small case series) that ischemic limb pain was diminished and that wounds healed.

Careful trial design is fundamental to collection of interpretable data. The Miyamoto et al study protocol was designed to diminish some of the highly variable rates of healing that are frequently observed in clinical practice and clinical investigation. Subjects were hospitalized for 1 month before and for 1 month after BM-MNC treatment. A prominent strength of this report is based on the relatively long length of follow-up, which spanned as little as 3 months and as much as 4 years.

From this design, the most prominent beneficial clinical outcome was an improvement in the visual analog scale pain score, supported by improvement in observed wound healing. Yet, despite this benefit, the ankle-brachial index did not demonstrate a directionally consistent improvement—it increased in 2, decreased in 3, and was unchanged in 4 patients. The ankle-brachial index may be an insensitive marker of therapeutic angiogenesis because transmission of conduit artery pressure to the ischemic limb end organ may not be captured by measurement of ankle blood pressure alone. In the absence of a measurement of skin blood flow or assessment of quantitative limb perfusion, the magnitude of induced angiogenesis is not known.

Most readers will note that the major trial design weaknesses of the present study include (1) a severely constrained sample size, (2) inclusion of multiple methods of performing the angiographic assessment, (3) variable application of TAO diagnostic and anatomic inclusion criteria (which may be particularly important with small study samples), and (4) lack of an independent adjudication of clinical events and inadequate collection of outcome safety data. These weaknesses are such major constraints that the simple conclusion that BM-MNC transplantation is associated with excellent short-term benefits is not well supported. In the absence of a control group, the treatment effect cannot be estimated, confounding standard care variables are not defined, and enrollment bias could lead to comparable rates of clinical improvement. Chronic pain often improves and wounds heal when treatment is offered by vascular specialists who treat individuals with CLI. In the absence of convincing angiographic improvement, measures of improved limb perfusion, or measurable increments in the ankle-brachial index, no therapeutic conclusion can be strongly defended. Other confounding variables may be critical, including the hospitalization and associated enforcement of abstinence from tobacco exposure. Could this be a study of hospital-based tobacco cessation for TAO?

**Adverse Event Reporting in Clinical Investigation**

The reporting of adverse events in clinical trials, in case series as well as large randomized trials, is a central element that informs investigators and the public about unanticipated clinical events and potential mechanisms that might be relevant to treatment. It is imperative that adverse events, even from small case series, be accurately reported. It has been estimated by the US General Accounting Office that as many as half of approved medications may have had at least one serious adverse reaction that was not recognized during the approval process.16 The accurate collection of adverse event data within a site, accurate communication to a trial database, transmission of this data to regulatory agencies, and publication of adverse events in the peer-reviewed literature are essential elements that may be deemed low priority within the challenging clinical research environment.17

The use of angiogenic therapies in cardiovascular research has been characterized by a reassuring safety profile thus far.18 Inasmuch as the balance of promise and hope versus anecdotal data and hype is so delicate for reassuring safety profile, there is a particular need for investigators and peer-reviewed mechanisms (journals and regulatory agencies) to focus attention on individual patients, small data sets, and larger exposures to these potentially therapeutic (or adverse) stem cell interventions.19

**Adverse Events From BM-MNC in CLI: Signal or Noise?**

The present report achieves its primary interest to clinician-investigators because of its proactive reporting of potentially high rates of major adverse events, including “death and unfavorable angiogenesis,” as defined by the authors. As reported in this trial and germane to future studies, these adverse event data are challenging to interpret. This study by Miyamoto et al can serve best as an unblinded and cautionary signal or noise report that describes a single death and other adverse outcomes associated with administration of BM-MNC.

Are there mechanisms by which BM-MNC transplantation might be responsible for these adverse events, including sudden cardiac death, as reported previously in the Therapeutic Angiogenesis using Cell Transplantation (TACT) study and now in this small TAO case series?111 There are theoretical bases for caution, derived from both preclinical data and early observations of clinical BM-MNC studies. First, migration of stem cells to sites distant from those targeted for therapy may alter the risk and benefit of treatment. Second, differentiation of BM-MNC may not be a fully directed process. Acceleration of new arterial plaque formation or creation of plaque instability has been demonstrated in preclinical models, as well as in-stent restenosis in the Myocardial regeneration and Angiogenesis in myocardial infarction with G-CSF and Intra-Coronary stem cell infusion (MAGIC) trial after granulocyte colony-stimulating factor and cell therapy administration.20 The development of an arteriovenous shunt might, in principle, result from a robust angiogenic response to BM-MNC implantation. Because both arteries and veins are known to be affected by the TAO inflammatory response, there is a potential disease-based mechanism by which stem cells might migrate to both arterial and venous vessels. The description of this vascular abnormality in the affected patient was not well described, however, and it could have existed before treatment but been discovered by the angiographic surveillance.
The Miyamoto et al report offers no information defining the mechanism of sudden death of the young patient who died at home. The absence of postmortem examination effectively blinds the authors, other investigators, and future potential study subjects from insight regarding the mechanism of risk, magnitude of risk, or any possible associated risks. It is impossible to evaluate whether this death is attributable to a direct (or a contributing) effect of BM-MNC transplantation.

The Implication to Future CLI Research
What conclusions can be drawn from this valuable case series? First, CLI represents the most severe manifestation of PAD that profoundly diminishes quality of life and global function and that is often associated with very high short-term mortality. Prompt recognition, vascular specialty referral, and revascularization are the current standard of care. Nevertheless, this care strategy is not always feasible, nor is it always effective. Evaluation of new pharmacological and angiogenic therapies would fill a real clinical need.

Second, the design and performance of CLI clinical investigations may require the creation of collaborative, multicenter networks that permit rapid subject accrual, use of control groups, clinical event committees to adjudicate outcomes, and detailed collection of adverse events to permit benefit and risk to be adjudicated by an independent data- and safety-monitoring board, investigators, and regulatory agencies. Finally, investigators, reviewers, and professional journals are now increasingly willing to report negative clinical trial outcomes or flagrantly adverse outcomes from well-performed case series.

Conclusion
There is a pressing unmet clinical need for new pharmacological or angiogenic treatments for CLI. The road from vascular biological principles underpinning the promise of cell therapy to an approachable therapy for individuals with CLI will require us to sustain optimism, share data generously, and evaluate risk and benefit diligently. When this is accomplished for CLI, this challenging manifestation of PAD will no longer be an orphan disease for which there is no effective medical treatment.

Disclosures
None.

References
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