# Stem Cell Therapy for Peripheral Arterial Disease

Leading researcher Douglas W. Losordo, MD, discusses the recent progress and future directions of this promising therapy.

***Endovascular Today:*** When and how did you begin researching stem cell therapy for peripheral arterial disease (PAD)?

**Dr. Losordo:** A group of investigators in the St. Elizabeth's Medical Center laboratory, which I was a part of, made an observation in the mid-1990s regarding the existence of a population of circulating mononuclear cells (MNCs) that had features consistent with progenitor or stem cells capable of forming new blood vessels. That was a very novel observation at the time because it really changed the way that we thought about new blood vessel formation in adults. Before that, we thought new blood vessels were formed in adults only by the sprouting of endothelial cells from pre-existing blood vessels. The demonstration that those circulating cells could form new blood vessels independently of pre-existing blood vessels highlighted the possibility that the adult circulatory system might contain building blocks for forming new blood vessels (Figure 1). We embarked on experiments beginning in the early to mid-1990s to test the ability of these progenitor cells in adults to help restore circulation. It was roughly 11 years ago that these investigations began in a large series of preclinical animal studies. At the time, we were in Boston and continued those studies in my lab. In December 2006, we came to Northwestern and continued those experiments.

***Endovascular Today:*** How would you describe the process by which stem cells aimed at treating PAD are derived?

**Dr. Losordo:** The cell that we have identified as having this potency to stimulate new blood vessel formation is

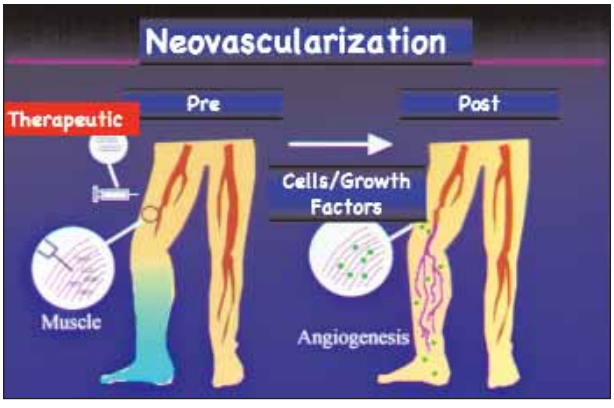


**Figure 1. Concept design for angiogenic therapy.**

referred to as a *CD34+ cell*. It is a very well-described cell in the hematology literature because it was noted many years ago that the CD34+ cell was capable of reconstituting bone marrow. The CD34+ cell also has the capability of stimulating the formation of new blood vessels; I mention that because there was a well-developed way of collecting and isolating these cells that had evolved over the years because of the hematologists' interest in using these cells for stem cell transplants. It involved the administration of an improved drug called *GCSF* (granulocyte colony-stimulating factor) for 5 days. The patients then undergo aphaeresis, which is a way to collect the MNC, a portion of the white blood cell from the circulation; it involved the insertion of an intravenous line and then processing the blood on an aphaeresis machine to collect the MNCs. The MNC fraction is then further purified to select for the CD34+ cells on another approved device, the Isolex 300i Magnetic Cell Selection System (Baxter Healthcare, Deerfield, IL), which selects for the

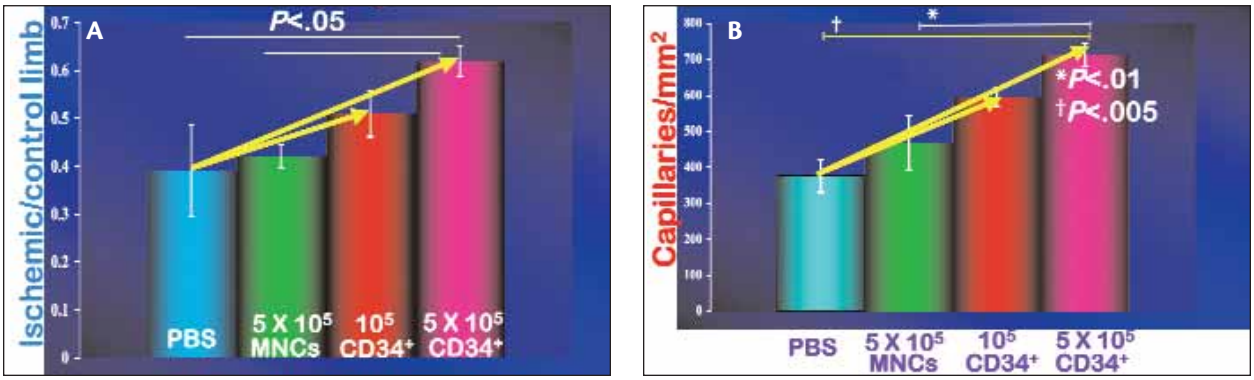


**Figure 2. Laser Doppler perfusion imaging ratio at day 28 of blood flow in the ischemic/control limbs (A). Capillary density in hind limb ischemia (B). (PBS, phosphate buffered saline.)**

CD34+ cells, and those CD34+ cells were used for injection into the ischemic muscle of patients with PAD.

***Endovascular Today:*** What kind of delivery techniques and technologies are used?

**Dr. Losordo:** Currently, the delivery technology for administration in patients with PAD is very low-tech. It is a straightforward, intramuscular injection, with the CD34+ cells being directly injected into the ischemic muscle. The preclinical studies demonstrated that direct injection of these cells into ischemic muscle led to recovery of circulation, formation of additional blood vessels, and improved blood flow into these ischemic muscles (Figure 2). That is the exact strategy that we are attempting in humans.

***Endovascular Today:*** Data from a study examining the use of stem cell therapy in critical limb ischemia (CLI) were published in the *Lancet* in 2002, showing promising clinical results in this early application. How has research of this therapy progressed since that time?

**Dr. Losordo:** That was a very exciting finding, and it involved the use of the entire bone marrow MNC population being directly injected into the ischemic muscle in patients with CLI. What has evolved since then, in this ongoing field of study, is that we have learned that there are populations of cells within that MNC population that appear to have increased potency and increased capability of forming new blood vessels. There are cells within that same mononuclear population that appear to inhibit the potency of those other cells, and research has been directed at better understanding the mechanisms by which these cells stimulate new blood vessel formation, which cells have that potency, and which cells inhibit that potency, so that we can properly select cells to get the maximum effect. The *Lancet* study from 2002 was simply proof of concept that these bone marrow-derived cells might have some therapeutic capability, and since then, much work has been done by many investigators to try to separate those populations so that we can end up with the best therapeutic option for that cell population.

Our work has led us to conclude that the CD34+ marker is useful for identifying cells with potency, and in fact, we have done comparison studies showing that if we use a CD34+ cell versus the entire MNC population, we do get a better result. I am sure we will be able to identify other populations that will have enhanced capabilities, but right now, it is a very stepwise process. Having developed the evidence that CD34+ is a marker for increased potency, we are proceeding with early-phase clinical studies.

***Endovascular Today:*** How would you describe the available literature and the clinical experience regarding the therapy?

**Dr. Losordo:** Most of the literature on CD34+ therapy for ischemic disease is preclinical and has come from our lab and a few others. Last year, we published the results of a pilot human clinical trial in patients with intractable angina and advanced coronary disease. I believe that the preclinical literature and our results really summarize the CD34+ cell literature. In the entire field of using progenitor cells or bone marrow-derived cells, for treating ischemic conditions, there is increasing literature that in total reflects that these cells appear to be safe to administer, especially autologous cells—the cells collected from the patients themselves. There seems to be safety associated with the administration of these cells, and I would say the data point to improved function and improved symptoms in patients who have been administered cells compared to placebo. It is an emerging field, so there is

not abundant literature; it is something that is just starting to be studied in controlled clinical trials.

*Endovascular Today:* Is it yet known which factors might predispose individual patients to respond favorably to stem cell therapy?

**Dr. Losordo:** We do not yet know exactly which factors drive the potency of these cells. There are data suggesting that in patients who have very advanced disease (eg, end-stage heart failure), the cells collected from those patients have reduced potency. This does not mean they have no potency, but it does present a challenge in terms of executing a strategy in which autologous cells are used, because the patients who need the most help may also be the ones whose progenitor cells are the least potent. Research aimed at determining ways to enhance the potency of cells is ongoing. Some of the important side studies being added to the early-phase clinical trials right now will attempt to identify subgroups of patients who respond better or worse, enabling us to increase our understanding of the cells and improve our clinical trial designs.

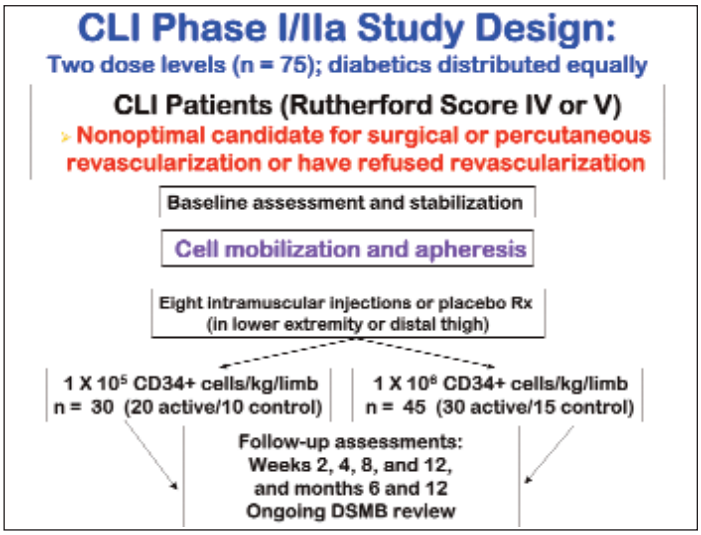


**Figure 3. Trial design of study with CLI patients.**

*Endovascular Today:* What can you tell us about the current clinical research underway at Northwestern?

**Dr. Losordo:** We have two studies that are currently ongoing; they are very similar and address patients at different stages of their PAD progression. The first is an NIH-funded study using autologous CD34+ cells, collected just as I described, in patients with severe intermittent claudication—patients who experience pain with walking but who do not yet experience pain at rest or have tissue breakdown. The cells are collected after mobilization and injected directly into the ischemic muscles in patients with intermittent claudication. Our more recent trial looks at patients with CLI (Figure 3), studying individuals whose disease has advanced to the point that they have pain at rest, many requiring narcotics for pain relief, or have tissue breakdown (nonhealing ulcers or the early stages of gangrene). Again, the strategy is somewhat identical: mobilization, collection of their progenitor cells, and injecting those CD34+ cells into the ischemic muscle. Five patients have been enrolled here at Northwestern in that study so far, with a plan for approximately 15 to 20 centers in the US enrolling patients in that 75-patient trial.

*Endovascular Today:* Is it difficult to enroll patients in a trial of an experimental therapy like this one?

**Dr. Losordo:** We have tried to precisely define the patient inclusion criteria for our studies, which include relatively small populations (75 patients). Our goal is to have as homogeneous a group of patients as possible, so that when we evaluate the response to therapy in treated versus control patients, we will have some chance of identifying a signal for bioactivity, if there is one. If the patient population is too heterogeneous, the variety will really nullify our ability to distinguish between the treatment groups. We have made it tough to enroll patients because we have narrowly defined our entry criteria, but it is not difficult in terms of finding patients who are interested. Many of the individuals who meet the study criteria have exhausted the conventional therapeutic options. They have undergone angioplasty, stenting, bypass surgery, and all that medicine has to offer, yet they are still left with this very severe disabling condition, which in a significant number of patients, will ultimately lead to amputation. These patients are highly motivated to try to find something that will eliminate their symptoms, improve profusion, and hopefully salvage the limb.

*Endovascular Today:* Do you believe that stem cell therapy will become a mainstream therapy for PAD?

**Dr. Losordo:** In the past decade, there has been an emergence of enhanced understanding or appreciation of just how important progenitor and stem cells are in the bodies of adults and how they are used on a fairly routine basis to maintain the integrity of tissue. It seems logical that if we can understand that biology and leverage it in patients in whom the natural mechanisms for tissue repair had failed, we will have an additional way to

treat patients to reverse some of the ravages of these diseases. I believe our understanding of stem cells will lead to new therapies for patients, probably involving the direct use of these cells for a period of time. Ultimately, just by understanding their biology, I think we will try to recapitulate some of the ability of stem cells, perhaps with medications.

***Endovascular Today:*** Are there any particular barriers to stem cell therapy as opposed to other device-related or medical methods that have been on the market?

**Dr. Losordo:** Yes, and they relate to the challenges involved in using a biological therapy such as this one versus the standard small-molecule drug approach. There is a well-established pharmacologic approach to testing a drug. It is quite a bit more challenging when a study involves the use of a biologic agent, for which dosing is not always the same as it would be for a pharmacologic agent; this is especially true in the case of an autologous cell, for which the potency of the cell itself may vary from individual to individual. We have to reinvent some methods for establishing safety and biopotency along the way. There are challenges in collecting and selecting these cells and identifying the particular cell type that we would like to use. Again, it is not a straightforward sort of chemical composition; in some ways, we are trying to identify a needle in a haystack within the patients themselves. There are many challenges facing this therapy, but I think the biological promise is driving the field forward in spite of those barriers.

***Endovascular Today:*** As stem cell therapy emerges as a prominent therapeutic option for PAD, which current vascular interventional specialty or specialties will be best suited to deliver it?

**Dr. Losordo:** The interesting thing about this type of therapy is that the technical aspect of delivering the therapy is fairly simple. Any medical practitioner could technically administer this therapy and perform an intramuscular injection. Traditionally, nurses perform intramuscular injections. However, the injections may be more involved here than just randomly injecting the cells; they may need to be targeted to a specific anatomy, and in that case, practitioners familiar with vascular anatomy who understand the way that ischemia derives due to arterial inclusive disease would probably be best suited. From a technical standpoint, intramuscular administration of these cells is pretty straightforward, so I do not see any potential barriers.

***Endovascular Today:*** Is there a specific time frame in which the treatment needs to take place for a CLI patient? How long does it take between deciding that the patient is going to receive this therapy to when they receive and see clinical benefits from it?

**Dr. Losordo:** Traditionally, when a new therapy—especially something that is really out of the mainstream—is evaluated, it is tested in the most severely ill patients because it is believed that the risk-to-benefit ratio is more acceptable in those very ill patients who are facing very severe situations. There is reason to believe we may actually have a better chance of attenuating the progress of disease rather than trying to reverse it once it reaches a very late, advanced stage. Our early indications in cardiac patients and others suggest that it probably takes a few months before a benefit will be observed in many patients. Therefore, I think there is at least a rationale to think about trying to administer these therapies as early in the disease process as we can or as early in the disease process as it seems appropriate and to expect that there would be some benefit to the patient. We are currently targeting patients with very advanced disease even though it is our understanding that it may take some time before the full manifestation of effect is evident.

***Endovascular Today:*** Do you think there will be a crossover in the future between identifying genetic biomarkers and then treating early-stage disease?

**Dr. Losordo:** I think you are absolutely right. One of the major benefits of studying these progenitor cells is it has given us a window into the pathobiology of some of these diseases. As we learn more and more about progenitor cell biology and how that relates to the advent and progression of disease, I think it will provide a biomarker that will allow us to identify patients who are at high risk for advanced disease and give us the opportunity to intervene, maybe at a time even before severe symptoms manifest, and therefore change the natural history of the disease. ■

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