Limb-saving medicines sought to prevent amputations

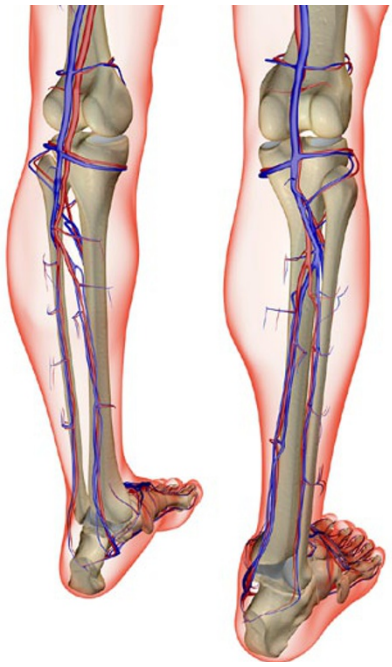
The number of lower limb amputations among diabetics has plummeted in recent years. According to a February report from the US Centers for Disease Control and Prevention, the rate of nontraumatic leg and foot amputations among adults with diagnosed diabetes fell by 65% between 1996 and 2008 (*Diabetes Care* **35**, 273–277, 2012). Still, the news isn't all good. Despite earlier diagnosis of foot and leg problems and better limb care, the diabetes-related amputation rate is still roughly eight times higher than the rate among healthy people, in part because arteries in the limbs of diabetics are more likely to become clogged with plaques—a condition known as peripheral artery disease (PAD).

But new therapies could be on the way for the tens of millions of people worldwide at risk of losing lower limbs each year. In December, the Russian Ministry of Healthcare and Social Development approved the world's first gene therapy product for the treatment of PAD. Meanwhile, Aastrom Biosciences of Ann Arbor, Michigan is on the cusp of launching a final-stage trial to test the company's cell therapy product ixmyelocel-T in 600 people with critical limb ischemia (CLI), the most severe form of PAD. This represents the first time that such a therapy for CLI has reached phase 3 testing anywhere in the world. "Cell-based therapies are the next frontier," says Mark Creager, a cardiovascular disease specialist at the Brigham and Women's Hospital in Boston.

Current treatment options for PAD usually involve medications to control blood pressure and blood sugar as well as lifestyle changes such as quitting smoking, exercising and eating right. If blood flow remains poor, physicians often take more drastic measures: they insert balloons or stents or reroute blood vessels to bypass any blockages. If the damage is too severe, then doctors resort to amputation.

To reverse or halt this trajectory, in the 1990s researchers began exploring noninvasive therapies that could trigger the formation of new blood vessels. For example, Jeffrey Isner, a cardiologist at the Tufts University School of Medicine in Boston, treated a 71-year-old woman with an ischemic right leg by inserting a balloon coated with plasmid DNA containing the gene encoding vascular endothelial growth factor (VEGF). The therapy prompted new blood vessels to form, a process known as angiogenesis, and blood flow improved (*Lancet* **348**, 370–374, 1996).

Data accumulated over the next ten years showed that angiogenic gene therapies had an acceptable safety profile. But as the therapies moved into larger trials, their efficacy proved underwhelming. In 2009, Cambridge, Massachusetts's Genzyme announced that its adenovirus therapy, designed to carry a gene encoding hypoxia-inducible factor 1α, failed to reduce leg pain in a phase 3 trial that included nearly 300 participants with PAD (*Circulation* **124**, 1765–1773, 2011). A year later, Sanofi, the French drug giant that bought Genzyme last year, announced that its gene therapy, a DNA plasmid carrying the gene encoding fibroblast growth factor 1, didn't have any effect on the number of deaths or amputations in a study of more than 500 people with CLI (*Lancet* **377**, 1929–1937, 2011).



MedicalRF / Photo Researchers, Inc.

In the loop: New drugs offer better circulation.

A daunting condition

No one is quite sure why these gene therapies failed. PAD is "a very daunting condition," says Douglas Losordo, a cardiologist who trained with Isner and now heads the new therapeutic development division at Baxter Healthcare in Deerfield, Illinois.

Angiogenesis is also a complex process. Perhaps therapies that focus on ramping up expression of a single gene are too simplistic, Losordo notes. Or maybe researchers haven't yet uncovered the right vector or delivery method. Either way, most researchers aren't ready to give up on gene therapy quite yet. Both Korea-based ViroMed and Ohio-based Juventas Therapeutics have gene therapy products for CLI in phase 2 development.

Then there's Neovasculgen, the *VEGF*-containing plasmid therapy product that gained approval in Russia late last year. In phase 3 testing—the results of which have not been published in English—researchers saw a doubling in pain-free walking distance in 120 people given Neovasculgen compared to 25 taking a placebo. Human Stem Cells Institute, the Moscow-based company behind the product, now plans to start testing the drug elsewhere. "We see great opportunities for the business development of Neovasculgen in the EU, as well as on the US and Asian markets," says general director Artur Isaev. Isaev also notes that the company will continue tracking trial participants for three years to determine whether the therapy also reduces the rate of amputations among people with CLI.

Meanwhile, researchers are also investigating more traditional medications to treat PAD and CLI, including anticoagulants, antiplatelet therapy and other drugs. Although some studies suggest that these medicines lower the risk of heart attack and stroke in people with PAD, they do not seem to produce long-term improvements in the ischemic limbs.

The strategy with the most commercial activity today involves using stem cells derived from bone marrow. "These cells are little factories of growth factors and cytokines that, at least theoretically, could help to stimulate new blood vessel growth," Losordo says.

Baxter and others, including Malaysia's Cytopeutics, India's Stempeutics and Germany's Apceth, are advancing their own cell-based therapies. But by far the most advanced is Aastrom. In phase 2 trial data reported in November at the American Heart Association's Scientific Sessions meeting in Orlando, Florida, the company showed that ixmyelocel-T, a mixture of macrophages and mesenchymal stem cells derived from patients' own bone marrow, reduced rates of complications such as amputations and gangrene by 62% compared to placebo.

The preclinical data suggest that ixmyelocel-T indirectly promotes blood vessel formation by secreting cytokines and growth factors that reduce inflammation. This may help explain the product's success, notes Aastrom chief executive Timothy Mayleben. "Blood flow is absolutely necessary, but it's not totally sufficient," he says. "We have to deal with the inflammation, as well."

Cassandra Willyard