Critical limb ischemia (CLI) is the most severe form of peripheral arterial disease and is defined as ischemic pain at rest for a period of ≥2 weeks, ulceration, and gangrene. Clinical progression is mostly unfavorable, with 25% of cases requiring amputation after a year of progression and is associated with high rates of mortality within 1 year of diagnosis. The leading risk factors for CLI are diabetes mellitus and age. In all cases, the restoration of perfusion through endovascular or surgical techniques should be considered as a first-line treatment. However, such treatment is possible in only 70% of cases. Furthermore, the durability of bypasses, particularly in cases of CLI, is poor and is not >50% to 70% after 3 years of surgery. Current treatment is thus based on local care of trophic problems, possibly combined with systemic antibiotic treatment. There is clearly a need to develop innovative and efficient approaches to restore blood flow and rescue limbs in patients with CLI. Therefore, many experimental studies and clinical trials have been performed with stem-/progenitor cell–based therapies thought to improve the patency of the affected region. Although meta-analysis indicates that intramuscular or systemic administration of bone marrow–derived cells or peripheral blood–derived cells is a feasible and relatively safe therapeutic approach for CLI, results from published clinical trials reveal some concerns about clinical proofs of efficacy precluding the use of cell therapy as a routine treatment for CLI.

These mixed results also challenge the scientific community to further elaborate the strategies for second, optimized generation of stem cell therapies. These should include the improvement of characteristic identities that benefit cell homing, engraftment, and survival. Similarly, the monitoring of stem cell isolation procedures, dose, route of administration, frequency, and timing of stem cell injection may affect the regenerative capacity of the ischemic tissue. Importantly, the positive outcome of the stem cell therapy relies on the fine-tuning of the delicate balance between the type of stem cells and the homeostasis of the targeted ischemic tissue. The cause and stage of the ischemic milieu should be taken into account because, for example, patients with severe limb ischemia characterized by Fontaine IV class may already be refractory to such cell–based strategies. Similarly, the cardiac microenvironment of a patient with acute myocardial infarction is different from the one enduring heart failure. Hence, the use of more appropriate stem cells with specific progeny for cardiovascular cell lineages and adapted tissue regeneration capacity should be proposed for efficient stem cell–based regenerative medicine.

An additional hurdle related to stem cells therapy is the use of the autologous approach. Most of the cardiovascular risk factors have been shown to reduce the availability of different subtypes of angiogenic cells, bone marrow, or circulating mononuclear cells and impair their function to varying degrees. In an elegant study, Katare et al² developed an interesting and innovative approach of allogeneic stem cell therapy using the CTX0E03 drug product, a manufactured product of consistent quality derived from a conditionally immortalized clonal human neural stem cell (hNSC) line. In an experimental model of CLI, they showed that treatment with hNSCs promoted neovascularization of the ischemic limb in immunodeficient animals as well as in immunocompetent CD-1 and streptozotocin-induced diabetic mice.² Comparison of the hNSCs with human mesenchymal stem cells indicated a similar improvement in blood flow. Histological studies in ischemic leg of immunocompetent mice during the first 7 days after treatment revealed short-term hNSC survival, transient elevation of early host muscle inflammatory and angiogenic responses, and acceleration of myogenesis.² However, grafted hNSCs mainly acquired an inflammatory phenotype, and the early transient increases in inflammatory cytokines, chemokines, and growth factor expression were observed in hNSC-treated ischemic muscle although classic inflammatory cell responses were not affected. Hence, the inflammatory phenotype and function of hNSCs are most likely induced by the implantation into the ischemic environment of immunocompetent mice. One can then speculate that hNSC phenotype and paracrine potential may be different in ischemic tissue of immunodeficient or diabetic mice. Alternatively, hNSCs induced complex and broad-spectrum changes in the expression of genes associated with inflammation in the ischemic host tissue of immunocompetent mice. It is then likely that the macro- and microenvironment in immunodeficient or diabetic mice may also affect the ability of hNSCs to activate host cells and orchestrate elements of ischemic tissue response and repair.

Immunodeficient animals are currently used to study the therapeutic ability of human stem cells. However, although the initial lack of immunoinflammatory response may emphasize the beneficial short-term effects of human stem cell therapy...
in these immunosuppressed models, the putative long-term rejection of these cells by the ischemic host tissue should not be underestimated. Interestingly, in this study, the immunogenic potential of hNSCs seemed to be low, and there was comparable efficacy in both nude and wild-type CD-1 mice, both at short-term and long-term, without any evidence of rejection of these cells from the recipient animals.\(^2\) This could be explained by a previous study that showed that CTX0E03 hNSCs do not express major histocompatibility complex II and do not promote a sustained host cellular response.\(^3\) The other well-known prototype of immunoprivileged cells suitable for allogeneic applications is human mesenchymal stem cells. However, although allogeneic human mesenchymal stem cells have been shown to be safe and effective as autologous cells in patients with ischemic cardiomyopathy,\(^4\) the long-term ability of allogeneic MSCs to preserve their function in the infarcted heart is limited by a biphasic immune response whereby they transition from an immunoprivileged to an immunogenic state after differentiation.\(^5\) Hence, long-term alterations in major histocompatibility complex–immune antigen profile and immunogenicity of grafted allogeneic cells, including hNSCs and human mesenchymal stem cells, should be carefully monitored in patients with ischemic diseases.

Finally, hNSCs also improved therapeutic revascularization in the ischemic leg of diabetic mice. In vitro, hNSCs give rise to all differentiated neural cells, but in vivo, the cells also deliver paracrine mediators that stimulate both angiogenesis and neurogenesis in animal models of stroke.\(^6,7\) Of great interest, the effect of neuropathy is of major importance on molecular and cellular mechanisms governing vessel growth in the diabetic setting.\(^5\) Alternatively, proangiogenic growth factors, such as vascular endothelial growth factor, have been shown to reverse ischemic and diabetic peripheral neuropathy.\(^9,10\) Although the effect of hNSCs on diabetic neuropathy and its putative involvement in tissue regeneration need to be confirmed, one can speculate that hNSC-based therapy targeting both vascular and neural elements may act on vasculopathy and neuropathy and may thus constitute an ideal approach for the treatment of diabetic patients with CLI. Overall, the study by Katare et al\(^2\) nicely demonstrates that hNSCs enter the race for cell-based therapeutic revascularization and supports the progression of hNSC product toward clinical application in patients with CLI.

**Disclosures**

None.

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When the Vessels Use Their Brain for Therapeutic Revascularization
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