STEM CELLS TRANSLATIONAL MEDICINE®

PERSPECTIVES

Cell Therapy for Chronic Limb-Threatening Ischemia: Current Evidence and Future Directions

MARTIN TERAA ,[a,b,*] HENDRIK GREMMELS,[a,*] JOEP G. J. WIJNAND,[a,b] MARIANNE C. VERHAAR[a]

[a]Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, The Netherlands; [b]Department of Vascular Surgery, University Medical Center Utrecht, Utrecht, The Netherlands

SUMMARY

Cell-based therapies have gained interest as a potential treatment method in cardiovascular disease in the past two decades, peripheral artery disease amongst others. Initial pre-clinical and small pilot clinical studies showed promising effects of cell therapy in peripheral artery disease and chronic limb-threatening ischemia in particular. However, these promising results were not corroborated in larger high quality blinded randomized trials. This has led to a shift of the field towards more sophisticated cell products, especially mesenchymal stromal cells. Mesenchymal stromal cells have some important benefits, making these cells ideal for regenerative medicine, e.g., potential for allogeneic application, loss of disease-mediated cell dysfunction, reduced production costs, off-the-shelf availability. Future high quality and large clinical studies have to prove the efficacy of mesenchymal stromal cells in the treatment of peripheral artery disease. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:842–846*

SIGNIFICANCE STATEMENT

Chronic limb-threatening ischemia is the most severe form of peripheral artery disease, and a considerable number of patients with this condition are not eligible for conventional treatment strategies. Therapies that aim at neovascularization might provide an escape for these patients. Initial clinical studies for first generation cell therapies were quite disappointing; however, more sophisticated and better defined cell therapies—mesenchymal stromal cells in particular—seem promising. This article describes the future perspectives and challenges of cell therapy in limb ischemia.

INTRODUCTION

Peripheral artery disease arises from atherosclerosis of major arteries, with a predilection for the lower limbs. In its most severe manifestation, occlusion of limb arteries reaches a point where metabolic demands of the tissue can no longer be met; this stage is termed chronic limb-threatening ischemia (CLTI) or critical limb ischemia (CLI). CLTI poses a great unmet need for novel treatments, as 20%–40% of the CLTI patients are not eligible for conventional revascularization, ultimately leading to amputation, associated with an immense medical and socio-economic burden [1–3]. No-option status in these patients is due to extensive and diffuse, often infrapopliteal, atherosclerotic lesions, comorbidity, and/or lack of a suitable bypass graft [4, 5]. Novel approaches that target neovascularization provide a potential solution for these no-option patients. Cell-based therapies seem the most promising [6], although initial enthusiasm has abated after negative results in the first generation of progenitor cell trials. Here we will provide a concise review on the available evidence on and future directions for cell therapy in CLTI. In that context we will also briefly address literature regarding cell therapy in myocardial infarction (MI) because objectives in both MI and CLTI trials are to enhance revascularization through cell therapy.

CELL THERAPY FOR CLTI—THE PRESENT STATE OF AFFAIRS

The rationale behind using progenitor cell therapy as a treatment for ischemic cardiovascular disease was motivated by the discovery that human blood contains progenitor cells that home to ischemic tissues [7] and augment angiogenesis [8]. Relatively soon thereafter, a first generation of progenitor cell trials have been conducted using bone marrow mononuclear

*Contributed equally.

Correspondence: Marianne C. Verhaar, M.D., Ph.D., Department of Nephrology and Hypertension, University Medical Center Utrecht, Heidelberglaan 100, HP F.03.227, 3584 CX Utrecht, The Netherlands. Telephone: +31 88 7557329; e-mail: m.c.verhaar@umcutrecht.nl Received February 3, 2018; accepted for publication July 3, 2018; first published August 1, 2018. http://dx.doi.org/10.1002/sctm.18-0025

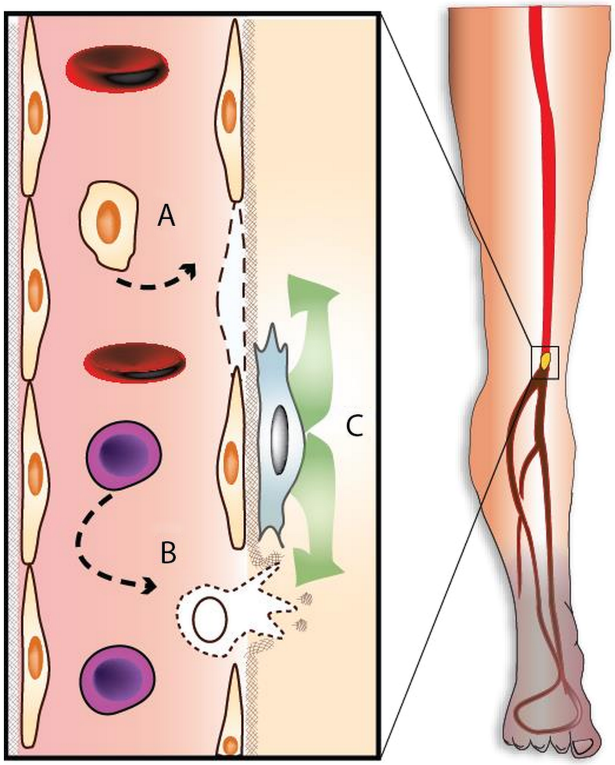

Figure 1. Different potential modes of action of cell therapy. **(A):** Direct angiogenesis through introduction of endothelial-like cells that will form new capillaries through vasculogenesis and fill endothelial defects. **(B):** Indirect angiogenesis through introduction of monocyte-like cells, that will remodel the extracellular matrix and will recruit and guide new endothelial sprouts. **(C):** Indirect angiogenesis through paracrine effects, including modulation of monocyte differentiation and recruitment of endothelial cells.

cells (BM-MNCs), a direct BM isolate which contains a variety of different cell-types, mostly from the hematopoietic line. The primary hypothesis was that BM, as the reservoir of hematopoietic stem cells, also contains endothelial progenitor cells (EPCs) [9]. These putative EPCs were initially thought to promote angiogenesis through the formation of new vessels [7] as they actively homed to ischemic areas after injection. Early, uncontrolled clinical studies using BM-MNCs were promising, but placebo-controlled trials gave conflicting results. A large, double-blind, placebo-controlled randomized trial by our group, the JUVENTAS trial, showed no treatment effects of BM-MNC administration over placebo [10], which was corroborated by a meta-analysis [11]. Similar results were obtained with BM-MNC therapy for other indications such as MI, where an aggregated study comprising over a 1,000 patients that were treated with BM-MNCs for MI failed to find a consistent positive effect [12].

Advancing insight into the biology of progenitor cells has in parallel, revealed that the mechanisms of effect involved in progenitor cell-therapy are different and more complex than initially thought. The use of cell surface markers to identify EPCs has been shown to be prone to isolation artifacts [13, 14], and several ontologically distinct cell populations display EPC markers. Furthermore it has been shown that BM-derived cells do not stably incorporate into newly formed vessels and only play an auxiliary role in neovascularization [15]. True vasculogenic ability has only been demonstrated for a single cell-type, designated the endothelial colony forming cell (ECFC) [16], which cannot be obtained from BM. While the auxiliary angiogenic effects of BM-MNCs have been demonstrated very consistently in animal models, it is likely that they only occur with BM isolates from comparatively young subjects without comorbidities [17, 18]. This restricts successful application of BM-MNCs in MI to specific subsets [19] of patients, and likely severely limits it in CLTI.

Collectively, these observations have precipitated a switch away from undefined raw cell isolates such as BM-MNCs, toward better-defined cell therapy products [20]. Whereas in the therapy for MI, the focus has shifted away from angiogenic cell therapy to cardiomyocyte regeneration [21], in CLTI the primary objective remains to augment angiogenesis.

Angiogenesis can be induced via different cell-based strategies (Fig. 1) by supplying endothelial-like cells, such as ECFCs directly, which will spontaneously organize into new vessels that integrate with the existing vasculature. Alternatively angiogenesis can be promoted indirectly by cells that secrete factors that remodel the extracellular matrix and recruit resident endothelial cells. In this category are circulating endothelial cells, which are of monocyte/myeloid origin, and may potentially act as bridging monocytes in angiogenesis [22]. Alternatively there are mesenchymal stromal cells (MSCs), which are of pericyte origin [23] and secrete a host of paracrine angiogenic and immunomodulatory factors [24]. However, it is debated that pericytes have the multilineage potential in vivo, which characterize MSCs in vitro and that the observed plasticity of MSCs result from manipulation ex vivo [25]. But irrespective of this it is likely that there are synergistic effects of combining these approaches, using a

Table 1. Overview of MSC trials in CLTI

Author	Year	*n*	Design	Injection sites	Total dose and source
Kim et al. [29]	2006	4	No control group	NM	1×10^6 allogeneic HLA matched UCB-MSCs
Dash et al. [30]	2009	24	Open label; control group (1:1 randomization)	NM	$45–60 \times 10^6$ autologous BM-MSCs
Lu et al. [31]	2011	41	Double blind study; randomly assigned treatment per leg; one leg treated with normal saline, the other treated with MNC or MSC	20	9.3×10^8 autologous BM-MSCs
Gupta et al. [32]	2013	20	Placebo controlled; double blind	40–60	2×10^6 allogeneic BM-MSCs per kilogram body weight
Gupta et al. [33]	2016	90	Nonrandomized; low dose, high dose or standard care	40–60	(1 or 2) $\times 10^6$ allogeneic BM-MSCs per kilogram body weight

Abbreviations: BM, bone marrow; HLA, human leukocyte antigens; MSCs, mesenchymal stromal cells; NM, not mentioned; UCB, umbilical cord blood.

combination of ECFCs and a supportive cell-type [26, 27]. At present, however, translation to a clinical product has proven difficult as ex vivo expansion of the above-mentioned cells requires specialty cell culture additives that are difficult and costly to obtain for clinical grade production [28]. For this reason only MSCs, have advanced to a second generation of clinical trials, as they can be relatively easily obtained from different tissues such as BM, placenta and adipose tissue and reproducibly expanded ex vivo (see Table 1 for overview).

MSC Therapy for CLTI

While MNC have proven to effectively enhance angiogenesis and neovascularization in preclinical studies, it has been suggested that the pro-angiogenic effect of MSCs is superior compared to MNCs in preclinical studies [34, 35]. In vitro and vivo studies have demonstrated that MSC can home to injured tissue and secrete beneficial factors that suppress inflammation and improve angiogenesis via paracrine pathways [36]. Several small exploratory clinical trials showed positive effects of MSCs in the treatment of CLTI compared to standard of care or placebo [30, 32, 37]. Clinical studies that directly compare BM-MSCs with MNCs for the treatment of CLTI are scarce. Only one study directly compared the two strategies in 41 diabetic CLTI patients, suggesting that MSC might be better tolerated and more effectively enhance perfusion and ulcer healing compared to MNC [31]. The disappointing results of clinical trials on MNC in CLTI, the promising effects of MSCs and several practical benefits of MSCs, in particular the potential for allogeneic application, have led to increased interest of MSCs as potential option for cell therapy in CLTI A similar switch is also observed for studies in cardiac disease [38].

MSCs, rather uniquely among transplanted cell grafts, are only minimally immunogenic [38] and display strong immunomodulatory properties [39]. This makes allogeneic application possible, at least in a single administration, as it is still unclear whether rejection occurs upon repeated administration [38]. At the present state of knowledge, allogeneic administration of MSCs is the most promising route to clinical application with the advantage of providing an off-the-shelf available product. An allogeneic product significantly reduces the burden on the patient, as patients will not have to undergo a (BM) harvesting procedure. Whereas in BM-MNCs it has been shown that patient-derived cell isolates show decreased pro-angiogenic effects [18], this does not necessarily apply to MSCs [40]. In a previous study we did not observe reduced angiogenic potency in CLTI MSC isolates in a murine hind limb ischemia model [40, 41]. In a clinical trial comparing efficacy of autologous versus allogeneic MSCs in non-ischemic dilated cardiomyopathy, however, allogeneic MSCs had a more favorable side-effect profile and a trend toward greater improvement in ejection fraction. Additionally the occurrence of serious adverse events was substantially lower in allogeneic then autologous MSCs; 28% versus 64%, respectively [38]. Furthermore, an advantage of allogeneic MSC therapy is that the (angiogenic) potency of the cell isolate can be tested in advance. Demonstrable potency will likely be of importance in the quality control of cell therapy products for clinical regulation [42]. As a single MSC isolate generally is sufficient to treat dozens of patients, a priori batch testing can conceivable improve clinical outcomes, provided that validated assays are developed [42, 43]. Last, allogeneic MSC administration is significantly less costly. Costs for expansion and quality testing of a BM isolate are high, which in autologous application were the per-patient cost, but which can be split over multiple patients in allogeneic application [44].

Current Challenges and Future Directions

Allogeneic application makes MSC-therapy interesting for commercial parties, as a defined cell product can be comparatively easily patented and produced by in-house companies, without the complications of harvesting donor material for each patient. Fifteen percentage of all clinical trials worldwide involving cell therapy are industry-sponsored and the vast majority of the remainder by leading academic centers. Additionally facilities and logistics involved in the development of cell therapy products are becoming more available and less expensive due to increased administration as standard of care or as investigational novel treatment in other diseases [45]. However, development of evidence-based accepted approaches remains challenging, due to high developmental costs, regulatory hurdles, and batch-per-batch product variation. Some of these factors may be less relevant for non-cellular

cell-based therapies, such as exosome-based therapies, which could make commercialization less difficult [46]. Another important CLTI-specific limiting factor is that, historically, the design of high-quality studies for the treatment of CLTI has proven notoriously difficult [47]. Improved clinical management and technical advances in revascularization approaches, endovascular interventions in particular, have led to a near doubling of 1-year amputation-free survival for CLTI patients since the first trials with BM-MNCs. Therefore larger and better-designed trials are required to determine the potential added value of novel therapies in CLTI [48, 49]. In the light of these considerations, small phase I/II [31, 32] or pragmatically designed studies [33] have provided valuable first indications about potential efficacy of MSCs in CLTI. However, at no point can they substitute evidence from placebo-controlled double-blind randomized trials. Public demand for cell-based therapies has been such, that smaller commercial parties are offering cell treatments in the absence of evidence—positive or negative—potentially putting patients at risk [50], leading to public discussions with respect to ethical issues regarding regenerative medicine approaches [51].

We therefore would encourage increased openness and standardization, both in the use of the investigational cell product and trial design. Convincing evidence for efficacy of MSC therapy will only come from well-designed randomized controlled trials using hard and clinically relevant outcomes, which would be ideally related with future imaging methods to evaluate collateralization and neovascularization. It seems increasingly unlikely that single investigative centers will achieve sufficient statistical power to show efficacy. International collaborative efforts and data sharing are necessary to push the field forward and maintain scientific integrity.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

References

1 Norgren L, Hiatt WR, Dormandy JA et al. Inter-society consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg 2007;45:S5–S67.

2 Barshes NR, Chamber JD, Cohen J et al. Cost-effectiveness in the contemporary management of critical limb ischemia with tissue loss. J Vasc Surg 2012 Oct;56:1015–1024.

3 Farber A, Eberhardt RT. The current state of critical limb ischemia: A systematic review. JAMA Surg 2016;151:1070–1077.

4 TASC Steering Committee, Jaff MR, White CJ et al. An update on methods for revascularization and expansion of the TASC lesion classification to include below-the-knee arteries: A supplement to the inter-society consensus for the management of peripheral arterial disease (TASC II). J Endovasc Ther 2015;22:663–677.

5 Setacci C, de Donato G, Teraa M et al. Chapter IV: Treatment of critical limb ischaemia. Eur J Vasc Endovasc Surg 2011;42:S43–S59.

6 Cooke JP, Losordo DW. Modulating the vascular response to limb ischemia: Angiogenic and cell therapies. Circ Res 2015;116:1561–1578.

7 Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–967.

8 Kalka C, Masuda H, Takahashi T et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci USA 2000;97:3422–3427.

9 Asahara T, Masuda H, Takahashi T et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85:221–228.

10 Teraa M, Sprengers RW, Schutgens REG et al. Effect of repetitive intra-arterial infusion of bone marrow mononuclear cells in patients with no-option limb ischemia: The randomized, double-blind, placebo-controlled Rejuvenating Endothelial Progenitor Cells via Transcutaneous Intra-arterial Supplementation (JUVENTAS) trial. Circulation 2015;131:851–860.

11 Peeters Weem SMO, Teraa M, de Borst GJ et al. Bone marrow derived cell therapy in critical limb ischemia: A meta-analysis of randomized placebo controlled trials. Eur J Vasc Endovasc Surg 2015;50:775–783.

12 Gyöngyösi M, Wojakowski W, Lemarchand P et al. Meta-Analysis of Cell-based CaRdiac stUdiEs (ACCRUE) in patients with acute myocardial infarction based on individual patient data. Circ Res 2015;116:1346–1360.

13 Prokopi M, Pula G, Mayr U et al. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. Blood 2009;114:723–732.

14 Gremmels H, Fledderus JO, van Balkom BWM et al. Transcriptome analysis in endothelial progenitor cell biology. Antioxid Redox Signal 2011;15:1029–1042.

15 Ziegelhoeffer T, Fernandez B, Kostin S et al. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ Res 2004;94:230–238. Epub 2003 Dec 4.

16 Yoder MC. Blood cell progenitors: insights into the properties of stem cells. Anat Rec A Discov Mol Cell Evol Biol 2004;276:66–74.

17 Heeschen C, Lehmann R, Honold J et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation 2004;109:1615–1622.

18 Dimmeler S, Leri A. Aging and disease as modifiers of efficacy of cell therapy. Circ Res 2008;102:1319–1330.

19 Zwetsloot PP, Gremmels H, Assmus B et al. Responder definition in clinical stem cell trials in cardiology: Will the real responder please stand up? Circ Res 2016 Aug 5;119:514–518.

20 Chavakis E, Koyanagi M, Dimmeler S. Enhancing the outcome of cell therapy for cardiac repair: Progress from bench to bedside and back. Circulation 2010;121:325–335.

21 Li TS, Cheng K, Malliaras K et al. Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere-derived cells. J Am Coll Cardiol 2012;59:942–953.

22 Urbich C, Heeschen C, Aicher A et al. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. Circulation 2003;108:2511–2516. Epub 2003 Oct 27.

23 Crisan M, Deasy B, Gavina M et al. Purification and long-term culture of multipotent progenitor cells affiliated with the walls of human blood vessels: Myoendothelial cells and pericytes. Methods Cell Biol 2008;86:295–309.

24 Caplan AI, Correa D. The MSC: An injury drugstore. Cell Stem Cell 2011;9:11–15.

25 Guimaraes-Camboa N, Cattaneo P, Sun Y et al. Pericytes of multiple organs do not behave as mesenchymal stem cells in vivo. Cell Stem Cell 2017;20:345–359.

26 Yoon CH, Hur J, Park KW et al. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. Circulation 2005;112:1618–1627.

27 Schwarz TM, Leicht SF, Radic T et al. Vascular incorporation of endothelial colony-forming cells is essential for functional recovery of murine ischemic tissue following cell therapy. Arterioscler Thromb Vasc Biol 2012;32:e13–e21.

28 Zeisberger SM, Zoller S, Riegel M et al. Optimization of the culturing conditions of human umbilical cord blood-derived endothelial colony-forming cells under xeno-free conditions applying a transcriptomic approach. Genes Cells 2010;15:671–687.

29 Kim SW, Han H, Chae GT et al. Successful stem cell therapy using umbilical cord blood-derived multipotent stem cells for Buerger's disease and ischemic limb disease animal model. Stem Cells 2006;24:1620–1626.

30 Dash NR, Dash SN, Routray P et al. Targeting nonhealing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells. Rejuvenation Res 2009;12:359–366.

31 Lu D, Chen B, Liang Z et al. Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb

ischemia and foot ulcer: a double-blind, randomized, controlled trial. Diabetes Res Clin Pract 2011;92:26–36.

32 Gupta PK, Chullikana A, Parakh R et al. A double blind randomized placebo controlled phase I/II study assessing the safety and efficacy of allogeneic bone marrow derived mesenchymal stem cell in critical limb ischemia. J Transl Med 2013;11:143.

33 Gupta PK, Krishna M, Chullikana A et al. Administration of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells in critical limb ischemia due to Buerger's disease: Phase II Study report suggests clinical efficacy. Stem Cells Translational Medicine 2016;6:689–699.

34 Arminan A, Gandia C, Garcia-Verdugo JM et al. Cardiac transcription factors driven lineage-specification of adult stem cells. J Cardiovasc Transl Res 2010;3:61–65.

35 Iwase T, Nagaya N, Fujii T et al. Comparison of angiogenic potency between mesenchymal stem cells and mononuclear cells in a rat model of hindlimb ischemia. Cardiovasc Res 2005;66:543–551.

36 Kinnaird T, Stabile E, Burnett MS et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 2004;94:678–685. Epub 2004 Jan 22.

37 Gupta PK, Krishna M, Chullikana A et al. Administration of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells in critical limb ischemia due to Buerger's disease: Phase II Study report suggests clinical efficacy. Stem Cells Translational Medicine 2017;6:689–699.

38 Hare JM, DiFede DL, Rieger AC et al. Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM trial. J Am Coll Cardiol 2017;69:526–537.

39 Le Blanc K, Tammik C, Rosendahl K et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematology 2003;31:890–896.

40 Gremmels H, Fledderus JO, Teraa M et al. Mesenchymal stromal cells for the treatment of critical limb ischemia: context and perspective. Stem Cell Res Ther 2013;4:140.

41 Gremmels H, Teraa M, Quax PH et al. Neovascularization capacity of mesenchymal stromal cells from critical limb ischemia patients is equivalent to healthy controls. Mol Ther 2014;22:1960–1970.

42 European Medicines Agency. Committee for Advanced Therapies (CAT). Reflection paper on stem cell-based medicinal products. EMA/CAT/571134/2009. 2011 Jan 14. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/02/WC500101692.pdf

43 Wijnand JGJ, Teraa M, Gremmels H et al. Rationale and design of the SAIL trial for intramuscular injection of allogeneic mesenchymal stromal cells in no-option critical limb ischemia. J Vasc Surg 2018;67:656–661. https://doi.org/10.1016/j.jvs.2017.09.026 Epub 2017 Dec 11.

44 Abou-El-Enein M, Bauer G, Medcalf N et al. Putting a price tag on novel autologous cellular therapies. Cytotherapy 2016;18:1056–1061.

45 Clarke D, Stanton J, Powers D et al. Managing particulates in cell therapy: Guidance for best practice. Cytotherapy 2016;18:1063–1076.

46 Rosca AM, Rayia DM, Tutuianu R. Emerging role of stem cells – Derived exosomes as valuable tools for cardiovascular therapy. Curr Stem Cell Ther 2017;12:134–138.

47 Teraa M, Conte MS, Moll FL et al. Critical limb ischemia: Current trends and future directions. J Am Heart Assoc 2016;5:e002938.

48 Benoit E, O'Donnell TF Jr, Kitsios GD et al. Improved amputation-free survival in unreconstructable critical limb ischemia and its implications for clinical trial design and quality measurement. J Vasc Surg 2012;55:781–789.

49 Iafrati MD, Hallett JW, Geils G et al. Early results and lessons learned from a multicenter, randomized, double-blind trial of bone marrow aspirate concentrate in critical limb ischemia. J Vasc Surg 2011;54:1650–1658.

50 Taylor PL, Barker RA, Blume KG et al. Patients beware: Commercialized stem cell treatments on the web. Cell Stem Cell 2010;7:43–49.

51 Niemansburg SL, Teraa M, Hesam H et al. Stem cell trials for cardiovascular medicine: ethical rationale. Tissue Eng Part A 2014 Oct;20:2567–2574.