Published in final edited form as:

Curr Opin Pharmacol. 2018 April ; 39: 27–34. doi:10.1016/j.coph.2018.01.005.

Cell therapy for peripheral artery disease

Nikolaos G Frangogiannis, MD*

The Wilf Family Cardiovascular Research Institute, Department of Medicine (Cardiology), Albert Einstein College of Medicine, Bronx NY

Abstract

Patients with severe peripheral artery disease (PAD) who are not candidates for revascularization have poor prognosis. Cell therapy using peripheral blood- or bone marrow-derived mononuclear cells, mesenchymal stem cells, or marker-specific subsets of bone marrow cells with angiogenic properties may hold promise for no-option PAD patients. Injected cells may exert beneficial actions by enhancing local angiogenesis (either through maturation of endothelial progenitors, or through secretion of angiogenic mediators), or by transducing cytoprotective signals that preserve tissue structure. Despite extensive research, robust clinical evidence supporting the use of cell therapy in patients with critical limb ischemia is lacking. Larger, well-designed placebo-controlled clinical trials did not support the positive results of smaller less rigorous studies. There is a need for high-quality clinical studies to test the effectiveness of cell therapy in PAD patients. Moreover, fundamental cell biological studies are needed to identify the optimal cell types, and to develop strategies that may enhance homing, survival and effectiveness of the injected cells.

Introduction

Lower extremity peripheral artery disease (PAD) is a major health burden, representing the third-leading cause of cardiovascular morbidity related to atherosclerotic disease after coronary disease and stroke. The prevalence of PAD rises sharply with age, affecting almost 20% of the US population at the age of 80^{1,2}. Epidemiologic studies have highlighted the global impact of the disease, suggesting dramatic recent increases in PAD prevalence in low and middle-income countries, and supporting the notion that we are faced with a global PAD pandemic, affecting more than 200 million men and women in both high-income countries and in the developing world³. Considering the mortality, morbidity and disability associated with PAD, there is an urgent need to develop new therapeutic strategies in order to prevent development and progression of the disease, and to treat life- or limb-threatening complications. Experimental studies and early stage clinical trials have suggested that cell therapy may be a promising new approach for patients with PAD⁴. The current review manuscript discusses the potential role of cell therapy approaches in the treatment of PAD.

*Address for correspondence: Nikolaos G Frangogiannis, MD, The Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, 1300 Morris Park Avenue Forchheimer G46B, Bronx NY 10461, Tel: 718-430-3546, Fax: 718-430-8989, nikolaos.frangogiannis@einstein.yu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The pathophysiologic basis of PAD

The clinical manifestations of PAD reflect the consequences of a mismatch between blood supply and demand^{5,6}. The typical symptom of PAD is intermittent claudication, a characteristic squeezing leg pain associated with walking and relieved by rest. In normal subjects, exercise is associated with marked increases in peripheral artery blood flow and limb oxygen uptake, driven by increased metabolic demand. In contrast, in PAD patients, fixed stenotic lesions in peripheral arteries limit blood flow, reducing the supply of the affected territory and leading to ischemia. Although the main cause of supply and demand disequilibrium in PAD patients is structural, excessive vascular tone due to activation of neurohumoral pathways, or impaired vasodilatory responses due to endothelial dysfunction may increase vascular resistance, further limiting blood flow in the extremity⁷.

Repetitive limb ischemia followed by reperfusion causes mitochondrial dysfunction in skeletal myocytes and triggers generation of reactive oxygen species (ROS), leading to chronic structural changes in the skeletal muscle. ROS-driven apoptosis of skeletal myocytes leads to a reduction in skeletal muscle mass and is accompanied by fatty infiltration, impaired peripheral nerve function and fibrosis^{8,6,9,10}. These pathologic alterations are associated with chronic skeletal muscle dysfunction and significant functional impairment. In a subset of patients, chronic ischemia follows an aggressive clinical course that culminates in the development of rest pain and significant tissue loss, a condition termed critical limb ischemia (CLI). Traditional treatment strategies in patients with CLI are focused on surgical bypass or endovascular interventions, aimed at restoring perfusion to prevent amputation of the affected limb¹¹. However, a significant percentage of CLI patients do not have revascularization options; these patients have poor prognosis and often require amputation.

Cell therapy as a therapeutic approach in PAD

Considering the limited treatment options for patients with severe PAD, the rationale for cell therapy approaches is sound. In patients with severe atherosclerotic disease of the native arterial circulation, administration of cell populations capable of activating an angiogenic program may result in formation of neovessels, improving perfusion of the affected limb. Increased blood supply may prevent ischemic episodes and may even contribute to restoration of normal skeletal muscle structure. It should be emphasized that any beneficial effects of cell therapy in PAD may not be necessarily due to incorporation of the cells into the vascular network, but may involve paracrine effects mediated through secretion of angiogenic mediators. Cell therapy may also activate yet unidentified cytoprotective and regenerative pathways that may improve limb function through effects independent of neovessel formation.

A growing body of experimental and clinical evidence suggests that cell-based therapy may hold promise in patients with severe PAD. Experimental investigations have used models of hindlimb ischemia to study the effectiveness of cell therapy approaches in promoting angiogenesis and in attenuating skeletal muscle injury. On the other hand most clinical studies investigating the effectiveness of cell therapy in patients with CLI are small phase I

or II clinical trials. Considering the variable approaches used by different groups, the wide range of cell types used, and the absence of standardized protocols for characterization of the cells and for evaluation of clinical outcome, there is substantial uncertainty regarding the effectiveness of various cell types in PAD patients.

The therapeutic potential of endothelial progenitor cells (EPCs)

The identification of EPCs, as bone marrow-derived progenitors, that home to sites of injury and may contribute to angiogenesis¹² provided a strong rationale for the use of cell therapy in PAD patients. It should be noted that, despite progress in understanding the mechanistic basis of the angiogenic response, the contribution of blood-derived progenitors in neovessel formation following injury remains controversial. In a mouse model of hindlimb ischemia, both marrow-derived and non-marrow derived endothelial progenitor populations have been implicated in formation of neovessels¹³. Despite the recent use of lineage tracing approaches in mouse models, the origin of neovascular endothelial cells in sites of injury remains controversial. Studies in the ischemic myocardium suggested a significant contribution of mesenchymal cells that undergo conversion into endothelial cells through a p53-dependent mechanism¹⁴. In contrast, other investigations suggested that practically all neovessels in the injured myocardium are derived from pre-existing endothelial cells, and not through lineage transdifferentiation¹⁵. It is plausible that the relative contributions of various cellular sources in the angiogenic response may be dependent on the pathophysiologic context and on the site of injury. Unfortunately, lineage tracing studies investigating the cellular origin of angiogenic vascular cells in ischemic skeletal muscle have not been performed.

Regardless of the origin of endogenous angiogenic endothelial cells in the ischemic limb, local injection of circulating endothelial progenitors would be expected to enrich the ischemic site with a pool of angiogenic cells, promoting neovessel formation and improving function. To achieve this goal, several different approaches have been used, injecting unselected or marker-specific mononuclear cells from the bone marrow, or the peripheral blood. These populations may contain a subset of bona fide endothelial progenitors that incorporate to the vascular network forming new vessels, and other cell types that may contribute to the angiogenic process by secreting cytokines, angiogenic growth factors, matrix metalloproteinases, matricellular proteins, or miRNA-containing exosomes (Figure 1)^{16,17,18}.

Bone marrow-derived mononuclear cells (BM-MNC) and peripheral blood-derived mononuclear cells (PB-MNC) in the treatment of PAD

Unselected mononuclear cells harvested, derived either from the bone marrow or the peripheral blood, represent a mixture of monocytes, non-hematopoietic stromal cells (including mesenchymal stem cells), and EPCs and have been used in both experimental models of limb ischemia and in patients with PAD. In the TACT (Therapeutic Angiogenesis using Cell Transplantation) study, injection with autologous BM-MNCs in the gastrocnemius of the ischemic limb reduced rest pain and increased transcutaneous oxygen pressure in patients with CLI; improvement was sustained for at least 24 weeks¹⁹. Over the last few years, several additional clinical trials suggested that intramuscular or intra-arterial

injections of BM-MNCs or PB-MNCs in patients with CLI are safe, and may reduce rest pain and improve ulcer healing (Table 1)^{20,21}. In many studies, improved clinical outcome was associated with objective evidence of enhanced perfusion. However, in most studies, effects on amputation rates did not reach statistical significance. Some of the larger, more rigorous, and well-designed studies failed to support the beneficial effects²², suggested by smaller nonplacebo controlled investigations²⁰. The conflicting findings may reflect the clinical improvement observed in placebo-treated patients²², and emphasize the importance of rigorous design, large population size and accurate blinding in order to test the effectiveness of therapy. The optimal cell type, concentration of cells and route of delivery remain unclear. Moreover the mechanistic basis for any protective effects remains obscure. Prolonged survival of the injected cells in the hostile environment of the ischemic limb has not been documented. Thus, it is unclear whether any benefit related to cell therapy is due to direct involvement of the cells in angiogenesis, or reflects paracrine effects that may include indirect stimulation of angiogenic pathways, modulation of inflammatory cascades, or cytoprotective actions on the ischemic limb.

Administration of marker-specific cells

Considering the functional and phenotypic heterogeneity of MNCs, identification of specific MNC subsets with angiogenic or cytoprotective properties represents a rational approach for further development of cell therapy strategies for PAD patients. Evidence suggests that CD34 may mark a subset of cells with angiogenic potential; a subset of CD34+ cells may be capable of differentiation to mature endothelial cells. In a randomized controlled pilot study, intramuscular injection of Granulocyte-Colony Stimulating Factor (G-CSF)-mobilized CD34+ cells in patients with CLI was safe and was associated with trends towards reduced amputation rates²³. Strategies using multicellular subsets of bone marrow-derived cells have also been tested. Ixmyelocel-T is an expanded population of bone marrow cells comprised predominantly of CD90+ mesenchymal cells and a subset of bone marrow macrophages. In a randomized phase 2 trial in patients with CLI, treatment with Ixmyelocel-T was safe, but did not significantly affect major amputation rates²⁴. Expression of high levels of the cytosolic enzyme aldehyde dehydrogenase (ALDH) has also been used to select a subset of angiogenic progenitors derived from bone marrow cells. In a randomized double-blind phase 2 clinical trial, intramuscular injection of cells with high ALDH activity did not improve peak walking time and perfusion in patients with claudication²⁵.

Mesenchymal stem cells (MSC)

Because of their potential for transdifferentiation and their potent effects in modulation of cell survival, inflammation and angiogenesis, MSCs are promising candidates for PAD cell therapy. A randomized controlled trial showed that infusion of autologous bone marrow-derived MSCs (BM-MSC) improved symptoms, accelerated ulcer healing and accentuated collateral blood vessel growth in diabetic patients with CLI²¹. Moreover, administration of allogeneic bone marrow-derived MSCs from healthy donors in patients with end-stage PAD was safe and well-tolerated, but did not have significant effects on amputation rates²⁶.

Appraisal of the clinical evidence on the effects of cell therapy in PAD

A recent meta-analysis of randomized, nonrandomized and noncontrolled studies for treatment of PAD suggested that although cell therapy did not affect all-cause mortality, it may have significantly improved the chances of amputation-free survival and ameliorated endpoints related to limb perfusion, pain and functional capacity in comparison with control treatment²⁷. However, efficacy of cell therapy on all endpoints was no longer significant in placebo-controlled studies and disappeared in randomized controlled trials with a low risk of bias²⁷. Thus, there is currently no robust evidence to support the effectiveness of cell therapy in patients with PAD.

PAD patients who are not candidates for revascularization strategies due to high risk, unfavorable vascular involvement, or failed endovascular approaches (no-option patients) represent a major therapeutic challenge and have a poor prognosis. It has been argued that, because there is no alternative to amputation in patients with end-stage CLI, cell therapy should be administered if available, even in the absence of robust evidence to support effects on amputation-free survival^{27,4}. However, implementation of expensive treatment strategies with minimal or no benefit to the affected patient population is of limited value, and may be harmful by reallocating healthcare resources, depriving other patient populations from highly effective therapies.

What are the reasons for the limited success of cell therapy in PAD patients? First, design of cell therapy approaches has been based predominantly on empiricism, and much less on sound cell biological insights. The lack of experimental animal models that recapitulate the pathophysiology of human PAD greatly complicates design of new strategies and limits our ability to test their effectiveness. Experimental evidence, when available, is often based on studies performed in young healthy animals that may not be relevant to the human PAD populations, typically comprised of older subjects with a high prevalence of diabetes, smoking and chronic atherosclerotic disease. Second, BM-MNCs and PB-MNCs typically used for treatment are highly heterogeneous, and may contain several different subsets with a wide range of effects. Specific subpopulations with protective angiogenic properties need to be defined. Third, the use of autologous cells in patients with comorbid conditions (such as diabetes) that may perturb their reparative and angiogenic properties may greatly limit effectiveness. Fourth, the fate of the cells following injection is unclear. Survival of the cells in the hostile environment of the ischemic limb may be limited; persistence of the cells in ischemic regions has not been consistently documented. Fifth, human PAD is characterized by anatomical heterogeneity, resulting in marked regional differences in the severity of ischemia and tissue damage. Refined administration strategies taking into account the regional distribution of ischemic lesions in PAD patients may be required to improve effectiveness.

There is little doubt that cell therapy in PAD treatment has a bright future. To achieve the full potential of this highly promising strategy, there is a need for a concerted effort to advance our knowledge on the fundamental cellular mechanisms of angiogenesis, while investing in robust clinical studies to test the most promising strategies in PAD patients.

Conclusions and future directions

The need for randomized double-blind placebo controlled studies to document any effects of cell therapy approaches in PAD patients cannot be overemphasized. The improvement observed in placebo-treated patients in PAD clinical trials^{22,28} emphasizes the need for rigorously designed and well-controlled studies in order to derive robust conclusions. Moreover, interpretation of the findings of clinical trials is dependent on introduction of endpoints for assessment of the cell biological consequences of the strategy. Assessment of cell homing and survival, and quantitative analysis of the effects of therapy on the vasculature in the ischemic area can provide critical information to understand the basis for success or failure, and to identify patient subpopulations with favorable responses.

Most importantly, we need to introduce new cell biological concepts in the design of cell therapy approaches. Dissection of the cell biological mechanisms of angiogenesis is critical to design an effective cell therapy approach for PAD patients. Understanding the phenotypic profile, properties, and mobilization mechanisms of endothelial progenitor populations and of mononuclear cell subsets with angiogenic properties is needed to define optimal cell types for therapy. Moreover, treatment with mediators that improve mobilization, homing and survival of endogenous progenitor cells may be useful to maximize benefit of cell therapy.

Chemokines are a family of chemotactic cytokines with an important role in leukocyte trafficking following ischemia²⁹. Several members of the chemokine family play essential roles in mobilization and migration of endogenous EPCs and may regulate their recruitment in ischemic sites^{30, 31}. Accentuation of chemokine signaling may be a promising strategy to enhance infiltration of the ischemic tissue with angiogenic cells. The CXC chemokine Stromal cell-derived factor (SDF)-1/CXCL12 is a key regulator of bone marrow cell mobilization and is critically involved in recruitment of progenitor cells in ischemic tissues^{32,33}. It has been suggested that in certain pathologic conditions, such as diabetes, hyperglycemia-mediated downmodulation of chemokine receptor expression in EPCs and other progenitor cells may reduce their homing in sites of injury, resulting in defective angiogenesis and impaired reparative responses. A recent study demonstrated that manipulation of EPCs to increase expression of the chemokine receptor CXCR7 (one of the receptors that mediate CXCL12 actions) improved outcome in a model of limb ischemia in diabetic mice, enhancing the angiogenic function of the cells³⁴. Other members of the chemokine family may act indirectly, increasing the angiogenic capacity of MNCs. The CX3C chemokine Fractalkine/CX3CL1 has been suggested to increase angiogenic potential of bone marrow-derived macrophages by accentuating expression of platelet factor-4/CXCL4³⁵. Pre-treatment of MNCs with mediators inducing an angiogenic program may stimulate their therapeutic potential in PAD. Moreover, genetic manipulations activating a pro-survival program in EPCs³⁶ or incorporation of matrix substrates that prolong survival and promote differentiation³⁷ may accentuate the beneficial actions of cell therapy.

Acknowledgments

Dr Frangogiannis' laboratory is supported by NIH grants R01 HL76246 and R01 HL85440, and by Department of Defense grants PR151134 and PR151029.

References

1. Criqui MH, Aboyans V. Epidemiology of peripheral artery disease. *Circ Res.* 2015; 116:1509–26. [PubMed: 25908725]
2. Allison MA, Criqui MH, McClelland RL, Scott JM, McDermott MM, Liu K, Folsom AR, Bertoni AG, Sharrett AR, Homma S, Kori S. The effect of novel cardiovascular risk factors on the ethnic-specific odds for peripheral arterial disease in the Multi-Ethnic Study of Atherosclerosis (MESA). *J Am Coll Cardiol.* 2006; 48:1190–7. [PubMed: 16979004]
3. Fowkes FG, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, Norman PE, Sampson UK, Williams LJ, Mensah GA, Criqui MH. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet.* 2013; 382:1329–40. [PubMed: 23915883]
4. Cooke JP, Losordo DW. Modulating the vascular response to limb ischemia: angiogenic and cell therapies. *Circ Res.* 2015; 116:1561–78. [PubMed: 25908729]
5. Hiatt WR, Armstrong EJ, Larson CJ, Brass EP. Pathogenesis of the limb manifestations and exercise limitations in peripheral artery disease. *Circ Res.* 2015; 116:1527–39. [PubMed: 25908726]
6. McDermott MM. Lower extremity manifestations of peripheral artery disease: the pathophysiologic and functional implications of leg ischemia. *Circ Res.* 2015; 116:1540–50. [PubMed: 25908727]
7. Ouriel K. Peripheral arterial disease. *Lancet.* 2001; 358:1257–64. [PubMed: 11675083]
8. Pipinos II, Judge AR, Zhu Z, Selsby JT, Swanson SA, Johanning JM, Baxter BT, Lynch TG, Dodd SL. Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. *Free Radic Biol Med.* 2006; 41:262–9. [PubMed: 16814106]
9. Ha DM, Carpenter LC, Koutakis P, Swanson SA, Zhu Z, Hanna M, DeSpiegelaere HK, Pipinos II, Casale GP. Transforming growth factor-beta 1 produced by vascular smooth muscle cells predicts fibrosis in the gastrocnemius of patients with peripheral artery disease. *J Transl Med.* 2016; 14:39. [PubMed: 26847457]
10. Koutakis P, Miserlis D, Myers SA, Kim JK, Zhu Z, Papoutsi E, Swanson SA, Haynatzki G, Ha DM, Carpenter LA, McComb RD, Johanning JM, Casale GP, Pipinos II. Abnormal accumulation of desmin in gastrocnemius myofibers of patients with peripheral artery disease: associations with altered myofiber morphology and density, mitochondrial dysfunction and impaired limb function. *J Histochem Cytochem.* 2015; 63:256–69. [PubMed: 25575565]
11. Teraa M, Conte MS, Moll FL, Verhaar MC. Critical Limb Ischemia: Current Trends and Future Directions. *J Am Heart Assoc.* 2016; 5
12. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* 1997; 275:964–7. [PubMed: 9020076]
13. Aicher A, Rentsch M, Sasaki K, Ellwart JW, Fandrich F, Siebert R, Cooke JP, Dimmeler S, Heeschen C. Nonbone marrow-derived circulating progenitor cells contribute to postnatal neovascularization following tissue ischemia. *Circ Res.* 2007; 100:581–9. [PubMed: 17272807]
14. Ubil E, Duan J, Pillai IC, Rosa-Garrido M, Wu Y, Bargiacchi F, Lu Y, Stanbouly S, Huang J, Rojas M, Vondriska TM, Stefani E, Deb A. Mesenchymal-endothelial transition contributes to cardiac neovascularization. *Nature.* 2014; 514:585–90. [PubMed: 25317562]
15. He L, Huang X, Kanisicak O, Li Y, Wang Y, Li Y, Pu W, Liu Q, Zhang H, Tian X, Zhao H, Liu X, Zhang S, Nie Y, Hu S, Miao X, Wang QD, Wang F, Chen T, Xu Q, Lui KO, Molkentin JD, Zhou B. Preexisting endothelial cells mediate cardiac neovascularization after injury. *J Clin Invest.* 2017; 127:2968–2981. [PubMed: 28650345]
16. Mathiyalagan P, Liang Y, Kim D, Misener S, Thorne T, Kamide CE, Klyachko E, Losordo DW, Hajjar RJ, Sahoo S. Angiogenic Mechanisms of Human CD34(+) Stem Cell Exosomes in the Repair of Ischemic Hindlimb. *Circ Res.* 2017; 120:1466–1476. This study highlights the role of exosome-shuttled proangiogenic miRNAs in mediating angiogenic effects of CD34+ stem cells. [PubMed: 28298297]
17. Raval Z, Losordo DW. Cell therapy of peripheral arterial disease: from experimental findings to clinical trials. *Circ Res.* 2013; 112:1288–302. [PubMed: 23620237]

18. Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY, Kim TY, Cho HJ, Kang HJ, Chae IH, Yang HK, Oh BH, Park YB, Kim HS. 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; 112:1618–27. [PubMed: 16145003]
19. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. Therapeutic Angiogenesis using Cell Transplantation Study I. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet*. 2002; 360:427–35. [PubMed: 12241713]
20. Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. *Diabetes Care*. 2005; 28:2155–60. [PubMed: 16123483]
21. Lu D, Chen B, Liang Z, Deng W, Jiang Y, Li S, Xu J, Wu Q, Zhang Z, Xie B, Chen S. 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. [PubMed: 21216483]
22. Teraa M, Sprengers RW, Schutgens RE, Slaper-Cortenbach IC, van der Graaf Y, Algra A, van der Tweel I, Doevendans PA, Mali WP, Moll FL, Verhaar MC. 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; 131:851–60. This well-designed randomized, double-blind, placebo controlled clinical trial found that repetitive intra-arterial infusion of autologous BM-MNCs did not reduce major amputation rates, when compared with placebo. Importantly, patients in the placebo group exhibited improvement in secondary outcomes, highlighting the significance of placebo-controlled design to test effectiveness of cell therapy approaches. [PubMed: 25567765]
23. Losordo DW, Kibbe MR, Mendelsohn F, Marston W, Driver VR, Sharafuddin M, Teodorescu V, Wiechmann BN, Thompson C, Kraiss L, Carman T, Dohad S, Huang P, Junge CE, Story K, Weistroffer T, Thorne TM, Millay M, Runyon JP, Schainfeld R. Autologous CDCTfCLII. A randomized, controlled pilot study of autologous CD34+ cell therapy for critical limb ischemia. *Circ Cardiovasc Interv*. 2012; 5:821–30. [PubMed: 23192920]
24. Powell RJ, Marston WA, Berceli SA, Guzman R, Henry TD, Longcore AT, Stern TP, Watling S, Bartel RL. Cellular therapy with Ixmyelocel-T to treat critical limb ischemia: the randomized, double-blind, placebo-controlled RESTORE-CLI trial. *Mol Ther*. 2012; 20:1280–6. [PubMed: 22453769]
25. Perin EC, Murphy MP, March KL, Bolli R, Loughran J, Yang PC, Leeper NJ, Dalman RL, Alexander J, Henry TD, Traverse JH, Pepine CJ, Anderson RD, Berceli S, Willerson JT, Muthupillai R, Gahremanpour A, Raveendran G, Velasquez O, Hare JM, Hernandez Schulman I, Kasi VS, Hiatt WR, Ambale-Venkatesh B, Lima JA, Taylor DA, Resende M, Gee AP, Durett AG, Bloom J, Richman S, G'Sell P, Williams S, Khan F, Gyang Ross E, Santoso MR, Goldman J, Leach D, Handberg E, Cheong B, Piece N, DiFede D, Bruhn-Ding B, Caldwell E, Bettencourt J, Lai D, Piller L, Simpson L, Cohen M, Sayre SL, Vojvodic RW, Moye L, Ebert RF, Simari RD, Hirsch AT. Cardiovascular Cell Therapy Research N. Evaluation of Cell Therapy on Exercise Performance and Limb Perfusion in Peripheral Artery Disease: The CCTRN PACE Trial (Patients With Intermittent Claudication Injected With ALDH Bright Cells). *Circulation*. 2017; 135:1417–1428. The study reports the effects of cell therapy with ALDH Bright cells in a population with claudication. Cell therapy was safe and well-tolerated, but did not affect peak walking time or magnetic resonance imaging-derived collateral counts and perfusion outcomes. Interestingly, there was no association between peak walking time and magnetic resonance imaging endpoints. [PubMed: 28209728]
26. Gupta PK, Chullikana A, Parakh R, Desai S, Das A, Gottipamula S, Krishnamurthy S, Anthony N, Pherwani A, Majumdar AS. 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. [PubMed: 23758736]
27. Rigato M, Monami M, Fadini GP. Autologous Cell Therapy for Peripheral Arterial Disease: Systematic Review and Meta-Analysis of Randomized, Nonrandomized, and Noncontrolled

- Studies. *Circ Res*. 2017; 120:1326–1340. Systematic review and metaanalysis of the studies evaluating efficacy of autologous cell therapy in patients with no-option PAD or CLI. Although the metaanalysis suggests that cell therapy reduced the risk of amputation, increasing ABI and decreasing rest pain, efficacy of cell therapy was no longer significant when analysis was limited to randomized placebo-controlled trials, and disappeared in randomized controlled studies with a low risk of bias. [PubMed: 28096194]
28. Peeters Weem SM, Teraa M, den Ruijter HM, de Borst GJ, Verhaar MC, Moll FL. Quality of Life After Treatment with Autologous Bone Marrow Derived Cells in No Option Severe Limb Ischemia. *Eur J Vasc Endovasc Surg*. 2016; 51:83–9. [PubMed: 26511056]
 29. Frangogiannis NG. Chemokines in ischemia and reperfusion. *Thromb Haemost*. 2007; 97:738–47. [PubMed: 17479184]
 30. Silvestre JS, Smadja DM, Levy BI. Postischemic revascularization: from cellular and molecular mechanisms to clinical applications. *Physiol Rev*. 2013; 93:1743–802. [PubMed: 24137021]
 31. Hristov M, Zernecke A, Liehn EA, Weber C. Regulation of endothelial progenitor cell homing after arterial injury. *Thromb Haemost*. 2007; 98:274–7. [PubMed: 17721606]
 32. Frangogiannis NG. Stromal cell-derived factor-1-mediated angiogenesis for peripheral arterial disease: ready for prime time? *Circulation*. 2011; 123:1267–9. [PubMed: 21403099]
 33. Segers VF, Revin V, Wu W, Qiu H, Yan Z, Lee RT, Sandrasagra A. Protease-resistant stromal cell-derived factor-1 for the treatment of experimental peripheral artery disease. *Circulation*. 2011; 123:1306–15. [PubMed: 21403096]
 34. Dai X, Yan X, Zeng J, Chen J, Wang Y, Chen J, Li Y, Barati MT, Wintergerst KA, Pan K, Nystoriak MA, Conklin DJ, Rokosh G, Epstein PN, Li X, Tan Y. Elevating CXCR7 Improves Angiogenic Function of EPCs via Akt/GSK-3 β /Fyn-Mediated Nrf2 Activation in Diabetic Limb Ischemia. *Circ Res*. 2017; 120:e7–e23. The study highlights the role of chemokine-driven mobilization and activation of EPCs in mediating their therapeutic effects in diabetic limb ischemia. [PubMed: 28137917]
 35. Park Y, Lee J, Kwak JY, Noh K, Yim E, Kim HK, Kim YJ, Broxmeyer HE, Kim JA. Fractalkine induces angiogenic potential in CX3CR1-expressing monocytes. *J Leukoc Biol*. 2017
 36. Kundu N, Domingues CC, Chou C, Ahmadi N, Houston S, Jerry DJ, Sen S. Use of p53-Silenced Endothelial Progenitor Cells to Treat Ischemia in Diabetic Peripheral Vascular Disease. *J Am Heart Assoc*. 2017; 6
 37. Lee SJ, Sohn YD, Andukuri A, Kim S, Byun J, Han JW, Park IH, Jun HW, Yoon YS. Enhanced Therapeutic and Long-Term Dynamic Vascularization Effects of Human Pluripotent Stem Cell-Derived Endothelial Cells Encapsulated in a Nanomatrix Gel. *Circulation*. 2017; 136:1939–1954. [PubMed: 28972000]
 38. Ozturk A, Kucukardali Y, Tangi F, Erikci A, Uzun G, Bashekim C, Sen H, Terekeci H, Narin Y, Ozyurt M, Ozkan S, Sayan O, Rodop O, Nalbant S, Sildiroglu O, Yalniz FF, Senkal IV, Sabuncu H, Oktenli C. Therapeutical potential of autologous peripheral blood mononuclear cell transplantation in patients with type 2 diabetic critical limb ischemia. *J Diabetes Complications*. 2012; 26:29–33. [PubMed: 22240264]
 39. Mohammadzadeh L, Samedanifard SH, Keshavarzi A, Alimoghaddam K, Larijani B, Ghavamzadeh A, Ahmadi AS, Shojaeifard A, Ostadali MR, Sharifi AM, Amini MR, Mahmoudian A, Fakhraei H, Aalaa M, Mohajeri-Tehrani MR. Therapeutic outcomes of transplanting autologous granulocyte colony-stimulating factor-mobilised peripheral mononuclear cells in diabetic patients with critical limb ischaemia. *Exp Clin Endocrinol Diabetes*. 2013; 121:48–53. [PubMed: 23329572]
 40. Szabo GV, Kovacs Z, Cserepes J, Daroczy J, Belkin M, Acsady G. Peripheral blood-derived autologous stem cell therapy for the treatment of patients with late-stage peripheral artery disease—results of the short- and long-term follow-up. *Cytotherapy*. 2013; 15:1245–52. [PubMed: 23993298]
 41. Raval AN, Schmuck EG, Tefera G, Leitzke C, Ark CV, Hei D, Centanni JM, de Silva R, Koch J, Chappell RG, Hematti P. Bilateral administration of autologous CD133+ cells in ambulatory patients with refractory critical limb ischemia: lessons learned from a pilot randomized, double-blind, placebo-controlled trial. *Cytotherapy*. 2014; 16:1720–32. [PubMed: 25239491]

42. Arai M, Misao Y, Nagai H, Kawasaki M, Nagashima K, Suzuki K, Tsuchiya K, Otsuka S, Uno Y, Takemura G, Nishigaki K, Minatoguchi S, Fujiwara H. Granulocyte colony-stimulating factor: a noninvasive regeneration therapy for treating atherosclerotic peripheral artery disease. *Circ J*. 2006; 70:1093–8. [PubMed: 16936417]
43. Prochazka V, Gumulec J, Jaluvka F, Salounova D, Jonszta T, Czerny D, Krajca J, Urbanec R, Klement P, Martinek J, Klement GL. Cell therapy, a new standard in management of chronic critical limb ischemia and foot ulcer. *Cell Transplant*. 2010; 19:1413–24. [PubMed: 20529449]
44. Benoit E, O'Donnell TF Jr, Iafrati MD, Asher E, Bandyk DF, Hallett JW, Lumsden AB, Pearl GJ, Roddy SP, Vijayaraghavan K, Patel AN. The role of amputation as an outcome measure in cellular therapy for critical limb ischemia: implications for clinical trial design. *J Transl Med*. 2011; 9:165. [PubMed: 21951607]
45. Iafrati MD, Hallett JW, Geils G, Pearl G, Lumsden A, Peden E, Bandyk D, Vijayaraghava KS, Radhakrishnan R, Ascher E, Hingorani A, Roddy S. 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; 54:1650–8. [PubMed: 22019148]
46. Walter DH, Krankenberg H, Balzer JO, Kalka C, Baumgartner I, Schluter M, Tonn T, Seeger F, Dimmeler S, Lindhoff-Last E, Zeiher AM, Investigators P. Intraarterial administration of bone marrow mononuclear cells in patients with critical limb ischemia: a randomized-start, placebo-controlled pilot trial (PROVASA). *Circ Cardiovasc Interv*. 2011; 4:26–37. [PubMed: 21205939]
47. Skora J, Pupka A, Janczak D, Barc P, Dawiskiba T, Korta K, Baczynska D, Mastalerz-Migas A, Garcarek J. Combined autologous bone marrow mononuclear cell and gene therapy as the last resort for patients with critical limb ischemia. *Arch Med Sci*. 2015; 11:325–31. [PubMed: 25995748]
48. Li M, Zhou H, Jin X, Wang M, Zhang S, Xu L. Autologous bone marrow mononuclear cells transplant in patients with critical leg ischemia: preliminary clinical results. *Exp Clin Transplant*. 2013; 11:435–9. [PubMed: 23477421]
49. Pignon B, Sevestre MA, Kanagaratnam L, Pernod G, Stephan D, Emmerich J, Clement C, Sarlon G, Boulon C, Tournois C, Nguyen P. Autologous Bone Marrow Mononuclear Cell Implantation and Its Impact on the Outcome of Patients With Critical Limb Ischemia- Results of a Randomized, Double-Blind, Placebo-Controlled Trial. *Circ J*. 2017; 81:1713–1720. [PubMed: 28603176]
50. Gupta PK, Krishna M, Chullikana A, Desai S, Murugesan R, Dutta S, Sarkar U, Raju R, Dhar A, Parakh R, Jeyaseelan L, Viswanathan P, Vellotare PK, Seetharam RN, Thej C, Rengasamy M, Balasubramanian S, Majumdar AS. 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 Transl Med*. 2017; 6:689–699. Of high significance. [PubMed: 28297569]

Figure 1.

Cell biological mechanisms responsible for the protective effects of cell therapy in peripheral artery disease. Most clinical studies have tested the effects of intramuscular or intra-arterial injections of bone marrow (BM) or peripheral blood-derived mononuclear cells (BM-MNC and PB-MNC respectively), marker-specific mononuclear cells, or bone marrow-derived mesenchymal stem cells (BM-MSK). These cells contain subsets of endothelial progenitor cells (EPCs) that may promote angiogenesis by incorporating into the vascular system. Injected cells may also act through paracrine actions by secreting angiogenic cytokines, growth factors, matrix metalloproteinases and mi-RNA-containing exosomes. Moreover, cell therapy may protect ischemic skeletal myocytes (SM), modulate inflammatory activity and inhibit fibrogenic pathways, preserving structure and function. Additional symbols: Ma, macrophage; F, fibroblast; EC, endothelial cell; P, pericyte.

Table 1

Findings of randomized controlled trials examining the effects of cell therapy in patients with Critical Limb Ischemia (CLI)

Type of cell therapy	Patient population	Follow-up	Main findings	Ref.
Autologous BM-MNCs (im). Autologous PB-MNCs (im).	Patients (84% males, 69% diabetic) with chronic limb ischemia, rest pain, non-healing ulcers (22%), gangrene (40%) without revascularization options. Group A: 25 patients with unilateral ischemia receiving BM-MNCs (vs. saline infusion in the less ischemic limb). Group B: 22 patients with bilateral ischemia, receiving BM-MNCs (in one limb) vs. PB-MNCs (in the other).	24w-3y	Cell therapy increased ankle-brachial index (ABI), reduced rest pain, improved walking time, and increased transcutaneous oxygen pressure (TcO ₂). Increases in ABI were higher in patients receiving BM-MNCs (vs. PB-MNCs). Follow-up suggested sustained improvement in leg pain and ulcer size for at least 2y.	19
Autologous G-CSF-mobilized PB-MNC (im)	28 diabetic patients (cell therapy 14, control 14) Gender: 18M, 10W. Mean age: 71y	3mo	Cell therapy reduced leg pain, reduced amputation rate (AR) (treatment: 0%, control: 20%), improved ulcer healing, increased perfusion, improved angiographic scores, and increased ABI.	20
Autologous G-CSF-mobilized PB-MNC (im)	40 diabetic patients (cell therapy 20, control 20). Gender: 29M, 11W. Mean age: 71.4y	12w	Cell therapy reduced pain, improved Fontaine score, improved ulcer healing, and increased ABI and TcO ₂ . No significant effects on AR (treatment: 15%, control: 25%).	38
Autologous G-CSF mobilized PB-MNCs (im)	21 diabetic patients (control 14, cell therapy 7) Mean age: 64y	3mo	Cell therapy improved walking ability, reduced AR (treatment: 0%, control: 50%), improved blood flow and increased ABI. There was a trend towards a reduction in ulcer size.	39
Autologous CD34+ cells (im, low and high dose protocols)	28 patients (control 12, low dose 7, high dose 9; 54% diabetic) Gender: 19M, 9F Mean age: 66y.	12mo	Cell therapy was safe and well-tolerated. There was a trend towards a reduction in AR (control: 75%, low dose: 43%, high dose: 22%)	23
Autologous expanded blood-derived "angiogenic cell precursors" (VesCell)	20 patients (control 10, cell therapy 10, 60% diabetic) Gender: 13M, 7F Mean age: 61.8y.	3mo-2y	Cell therapy reduced AR (major amputations in the treatment group: 0% at 3mo and 30% at 2y and in the control group 60% at 3mo and 2y), increased ABI at 3mo and 2y, and increased TcO ₂ .	40
Autologous CD133+ cells sorted from G-CSF-mobilized PB-MNCs	10 patients (2:1 randomization) Gender: 8M, 2W. Mean age: control 85y, treatment 65y.	12mo	Subject enrollment was suspended due to a high rate of mobilization failure. Cell therapy was associated with non-significant trends towards lower AR (control: 66%, treatment: 14%), reduced walking impairment and improved quality of life	41
Autologous BM-MNCs (im)	25 patients (treatment group n=13, control n=12). Age: treatment 62y, control 68y.	1mo	Cell therapy improved rest pain, and increased ABI and TcO ₂ . There were no reports of amputations in control or treatment groups.	42
Autologous bone marrow aspirate concentrate (im)	96 patients Cell therapy: n=42 (36M, 6F), Control: n=54 (42M, 12F) Age: treatment 66y, control 64y. Diabetes: treatment 88.1%, control 98.2%.	120d	Cell therapy reduced rest pain, decreased AR (treatment 21%, control: 44%), and increased ABI and TcO ₂ . The bone marrow concentrate of patients who failed therapy exhibited lymphopenia.	43

Type of cell therapy	Patient population	Follow-up	Main findings	Ref.
Autologous bone marrow aspirate concentrate (im)	48 patients. Treatment n=34 (23M, 11F), control n=14 (9M, 5F). Age: treatment 72.5y, control 65.7y. Diabetes: treatment 52.9%, control 42.8%.	6mo	Cell therapy was well tolerated. There were trends towards reduced AR (treatment: 17.6%, control: 28.6%), reduced pain, increased ABI and better quality of life in the cell therapy group.	44,45
Autologous BM-MNCs (im) Autologous BM-MSCs (im)	41 diabetic patients, mean age: 64y BM-MNC group (n=21 limbs, males: 42%), BM-MSC group (n=20 limbs, males: 39%), Normal saline (n=41 limbs).	24w	Administration of BM-MNCs and BM-MSCs was well-tolerated. Cell therapy reduced AR (0% in BMMSC and BM-MNC group, vs. 16.2% in control group) and improved rest pain. Ulcer healing was accelerated in the BM-MSC group in comparison with BM-MNC patients.	21
Autologous BM-MNC (ia)	39 patients (treatment n=19, control n=20) Age: treatment 64.4y, control 64.5y. Diabetes: treatment 53%, control 48%. Male: treatment 84%, control 62%.	3mo	Cell therapy did not significantly affect AR (treatment 21%, control 5%) and did not significantly increase ABI. However, cell therapy improved ulcer healing and reduced rest pain.	46
Autologous BM-MNCs (im)+ VEGF gene therapy	32 non-diabetic patients. Treatment: n=16, 11M 5W, mean age 66.8y. Control: n=16, 10M, 6W, mean age 68.3y.	3mo	No statistically significant difference in AR between groups (treatment: 25%, control: 50%). Only patients enrolled in the treatment group exhibited increased ABI (75% of treated patients) and ulcer healing (69% of treated patients).	47
Autologous BM-MNCs (im)	58 patients BM-MNC: (n=29, 22M, 7F), Placebo: (n=29, 23M, 6F). Diabetes: treatment 44.8%, control 41.4%. Mean age: treatment 61y, control 63y.	6mo	Cell therapy improved rest pain and ulcer healing and increased ABI. There was no significant difference in the rate of major amputations (treatment 10%, control 17%).	48
Autologous expanded BM-MNCs, containing CD90+ cells and a subset of macrophages (Ixmyelocel-T)	72 patients Treatment: n=48, 34M 14F. Control: n=24 14M 10F. Diabetes: treatment 44%, control 63%. Age: treatment 69.2y, control 67.3y.	12mo	Cell therapy was well-tolerated, but did not affect AR (control: 25%, treatment 21%).	24
Repetitive (3 times in a 3-week interval) autologous BM-MNC (ia)	160 patients. Treatment: n=81 (57M, 24F), control: n=79, (51M 28F). Age: treatment 69y, control 65y. Diabetes: treatment 36%, control 39%.	6mo	Cell therapy had no effects on AR (control 13%, treatment 19%), rest pain, ABI and TcO ₂ .	22
Autologous BM-MNC (im)	38 patients (treatment n=18, placebo n=20). Diabetes: treatment 55%, control 35%.	6–12mo	Cell therapy has no effects on AR (placebo: 26%, treatment: 18%). Cell therapy and control groups had comparable improvements in rest pain and in TcO ₂ .	49
Allogeneic expanded BM-MSC derived from healthy donors (im)	20 patients. MSC group (n=10), placebo (n=10) Patients had either atherosclerotic disease or thrombangitis obliterans	6mo	Cell therapy was safe and increased ABI. There were no significant effects on AR.	26
Allogeneic expanded BM-MSC derived from healthy donors (high and low dose groups - im)	90 patients with CLI due to thrombangitis obliterans. Control (n=18), low dose (n=36), high dose (n=36). Age range: 38–42y.	6mo	AR was comparable between groups. High dose BM-MSCs reduced rest pain and improved ulcer healing. Both low and high-dose groups exhibited improved quality of life scores.	50

CLI, critical limb ischemia; Ref., Reference; G-CSF, granulocyte-colony stimulating factor; AR, amputation rate; ABI, ankle-brachial index; TcO₂, transcutaneous oxygen pressure; PB-MNC, peripheral blood-derived mononuclear cells; BM-MNC, bone marrow-derived mononuclear cells; BM-MSC, bone marrow-derived mesenchymal stem cells; im, intramuscular; ia, intra-arterial; M, men; W, women; mo, months; w, weeks; d, days; y, years