Concise Review: Therapeutic Potential of Adipose Tissue-Derived Angiogenic Cells

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ABSTRACT

Inadequate blood supply to tissues is a leading cause of morbidity and mortality today. Ischemic symptoms caused by obstruction of arterioles and capillaries are currently not treatable by vessel replacement or dilatation procedures. Therapeutic angiogenesis, the treatment of tissue ischemia by promoting the proliferation of new blood vessels, has recently emerged as one of the most promising therapies. Neovascularization is most often attempted by introduction of angiogenic cells from different sources. Emerging evidence suggests that adipose tissue (AT) is an excellent reservoir of autologous cells with angiogenic potential. AT yields two cell populations of importance for neovascularization: AT-derived mesenchymal stromal cells, which likely act predominantly as pericytes, and AT-derived endothelial cells (ECs). In this concise review we discuss different physiological aspects of neovascularization, briefly present cells isolated from the blood and bone marrow with EC properties, and then discuss isolation and cell culture strategies, phenotype, functional capabilities, and possible therapeutic applications of angiogenic cells obtained from AT. STEM CELLS TRANSLATIONAL MEDICINE 2012;1:658–667

INTRODUCTION

Inadequate blood supply to tissues is a leading cause of morbidity and mortality today. Narrowing of the vessels of the arterial tree may be caused by a range of diseases and environmental factors, with slightly different sets of etiological factors affecting large and medium-sized arteries and arterioles [1]. Obstructions of large and medium-sized arteries are frequently amenable to surgical or endovascular repair procedures. However, some of these procedures require the replacement of obstructed vessels with patent vessels obtained from other parts of the patient. The availability of redundant vessels is obviously limited. This has opened up a very active research field where the aim is to create arterial vessels by tissue engineering using biomaterials and autologous cells [2]. Further down the arterial tree, ischemic symptoms caused by obstruction of arterioles and capillaries are currently not treatable by replacement or dilatation procedures [3]. These symptoms most commonly occur in the limbs, where the disorder is called peripheral vascular disease (PVD), and in the heart, where arteriolar obstruction is one of the causes of refractory angina pectoris. Attempts to treat PVD using angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), or hepatocyte growth factor (HGF) have been disappointing [4]. Over the past decade, researchers have turned to the use of cells in attempts to produce neovascularization of ischemic tissues [5, 6]. In this concise review we briefly describe the mechanisms involved in de novo blood vessel formation, summarize the results of clinical trials using cells to treat microvascular tissue ischemia, and then focus on adipose tissue as a source of cells with therapeutic angiogenic potential.

NEOVASCULARIZATION-DE NOVO BLOOD VESSEL FORMATION

Formation of new blood vessels is a complex and integrated process that is not yet completely understood. It is important during embryological organogenesis, in the course of organ growth after birth, in the course of restoration of blood supply to ischemic tissues, and in the establishment of blood supply to tumors [5]. Neovascularization is the term used for the physiological processes of angiogenesis, vasculogenesis, and arteriogenesis, which represent different aspects of this complex process (Fig. 1). In angiogenesis, new microvessels are generated from pre-existing vasculature by the proliferation and migration of endothelial cells (ECs). These vessels play an important part in the repair mechanism of damaged tissues [5]. Hypoxia is an important stimulus for the expansion of the vascular bed, particularly through the effects of hypoxia-inducible factors (HIFs) [7]. HIFs upregulate angiogenic factors such as VEGF, which
stimulate both physiological and pathological angiogenesis. Vasculogenesis, on the other hand, refers to the formation of blood vessels by the recruitment and differentiation of undifferentiated endothelial progenitor cells at the site of new vessel growth [5]. This process is regulated by growth factors such as VEGF, FGF, transforming growth factor, and angiopoietin-1 and by their receptors, including VEGF receptor 1 (VEGFR1/FLT1), VEGFR2 (KDR/FLK1), and Tie-2 [8]. Until recently, the term vasculogenesis was used only to describe blood vessel formation in the embryo. However, this process has now also been shown to contribute to adult blood vessel formation [9, 10]. Arteriogenesis involves the expansive growth of collateral arteries by sprouting of pre-existing vessels to form collateral bridges between arterial networks via the migration and proliferation of ECs and SMCs. Growth factors and cytokines released endogenously in response to tissue ischemia act to promote neovascularization. Abbreviations: Ang 1, angiopoietin-1; EC, endothelial cell; EPC, endothelial progenitor cell; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; SMC, smooth muscle cell; VEGF, vascular endothelial growth factor.

**Figure 1.** The processes of angiogenesis, vasculogenesis, and arteriogenesis. New microvessels are generated from pre-existing vasculature by the proliferation and migration of mature ECs in the classic process of new vessel growth, angiogenesis. Vasculogenesis involves participation of undifferentiated EPCs, which circulate to sites of new vessel growth, where they differentiate into mature ECs. Arteriogenesis involves the expansive growth of collateral arteries by sprouting of pre-existing vessels to form collateral bridges between arterial networks via the migration and proliferation of ECs and SMCs. Growth factors and cytokines released endogenously in response to tissue ischemia act to promote neovascularization. Abbreviations: Ang 1, angiopoietin-1; EC, endothelial cell; EPC, endothelial progenitor cell; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; SMC, smooth muscle cell; VEGF, vascular endothelial growth factor.

Endothelial cells in bone marrow and peripheral blood

During embryogenesis, endothelial and hematopoietic lineages have common lineage precursors [11]. These common precursors, sometimes called hemangioblasts, are located in embryonic vessel walls at least through part of embryonic development.
Adipose tissue as a source of cells with angiogenic potential

Adult adipose tissue (AT) is one of the largest and most plastic tissues in the body. AT is the source of a range of hormones and cytokines, is a main reservoir of energy, and frequently goes through periods of expansion and shrinkage. Not surprisingly, AT is one of the most highly vascularized tissues in the body. A very close anatomical and physiological relationship has been demonstrated in AT between blood vessels, perivascular cells, and adipocyte precursor cells [21]. Mesenchymal stromal cells (MSCs) may differentiate to adipocytes and may be the earliest adipocyte precursor cells in AT. However, MSCs also have a role as perivascular cells, thus stabilizing new blood vessels [22, 23]. At the same time, the vasculature may have a causal role in the physiological functions of AT by controlling the number of microvessels and by remodeling existing vessels. Indeed, angiogenesis has been shown to be of great importance for the modulation of adipogenesis and obesity [24]. Thus, AT is an easily available, sometimes greatly superfluous tissue where new blood vessels are constantly being made in adult life.

The availability of ample amounts of tissue has generated a search for interesting and useful cell populations within AT. For this, variable amounts of liposuction material can be collected under local anesthesia by minimally invasive interference. After removal of blood from the liposuction material, the connective tissue keeping the remaining tissue together is digested using collagenase. Adipocytes, which make up the majority of the bulk of this tissue, are separated from other cells by gentle centrifugation. The pellet recovered from this centrifugation step is called the stromal vascular fraction (SVF) of AT. Analysis of SVF revealed that AT is a source of cells with multilineage differentiation potential [25, 26]. However, it soon became clear that SVF is, in fact, a heterogeneous population of cells. Several markers can be used to distinguish the populations contained within SVF, but the most useful may be CD31 and human leukocyte antigen (HLA) DR, which are molecules normally expressed on ECs. Upon phenotypic characterization of SVF, these molecules separate SVF cells into two populations: those coexpressing CD31 and HLA DR, approximately 20%–40% of the SVF, and those expressing neither of these [27]. After some weeks of in vitro culture, the plastic-adherent CD31⁺ HLA DR⁺ population expressed surface markers typical of MSCs [28]. These cells are frequently called adipose tissue-derived stem cells (ADSCs or ASCs), although we prefer to call them adipose tissue-derived mesenchymal stromal cells (AT-MSCs) to mark their ontogenetic relationship to bone marrow (BM) MSCs and at the same time distinguish them from other stem cells that may be found within the SVF. Transcription profiling analysis shows that the CD31⁺ HLA-DR⁺ cells within SVF overexpress transcripts associated with both arterial and venous endothelium and mostly resemble microvascular cells [27]. Under the culture conditions used in this study, which were optimized for MSC culture with Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium and no gelatin coat on the plastic surface, the CD31⁺ SVF cells did not proliferate in vitro. Later we successfully isolated and in vitro expanded CD31⁺ cells from AT using other cell culture conditions and showed that these were indeed bona fide ECs [29].

Neovascularization potential of AT-MSCs

AT-MSCs may be obtained in high numbers from SVF by removal of CD31⁻ cells [27]. In fact, the frequency of MSCs within mononuclear cells from AT is at least 500-fold higher than in mononuclear cells from bone marrow [30], yielding sufficient numbers of
uncultured AT-MSCs to allow phenotypic and molecular characterization. Comparisons of uncultured AT-MSCs with their culture-expanded offspring showed that plastic-adherent cell culture induced considerable differences in gene expression and surface molecules [27]. Most notable, perhaps, was the expression of CD34 by practically all the uncultured AT-MSCs. This molecule, which is also expressed at low levels by most ECs, was lost over the first few passages of plastic-adherent cell culture. Thus, culture-expanded adipose-derived stem cells appear as a relatively homogeneous population. They adhere to the definition of MSCs provided by the International Society for Cell Therapy based on their phenotype (CD73+, CD90+, CD105+, CD11b/CD14+, CD19/CD73b, CD34+, CD45+, HLA DR−); their plastic-adherent properties; and their multipotent differentiation potential to adipogenic, chondrogenic, and osteogenic lineages [28]. Based on their differentiation capabilities, AT-MSCs are being used today for breast re-establishment and enlargement surgery and for tissue engineering of cartilage and bone. However, their role in therapeutic neovascularization procedures is still unclear [31].

The CD31+ population of SVF expresses very much lower levels of mRNAs encoding EC molecules such as CD144, CD31, vWF, VEGFR2, and VEGFR1 than does the uncultured CD31+ subset of SVF [27]. At the same time, the CD31− cells secrete a range of soluble factors. Some, such as VEGF and HGF, are known to promote neovascularization [32]. Using the mouse ischemic hind limb model to determine the neovascularization potential, the stromal cell fraction of mouse and human SVF was found to improve angiogenesis mainly by the secretion of angiogenic growth factors [33]. Similar mechanisms were shown to act when rat AT-MSCs protected skin flaps against ischemia-reperfusion injury [34]. However, other investigators showed that injected AT-MSCs improved the ischemic score also by differentiation to CD31+ ECs within ischemic tissues [35–37]. Yet other studies failed to demonstrate the differentiation of adipose-derived cells toward the endothelial lineage [38], possibly because of differences in passage number and culture conditions. Then, in 2008, a number of studies appeared that suggested that MSC populations derive from blood vessel walls and that they may be identical to the pericytes [22, 39, 40]. A landmark paper by Crisan et al. described the in situ and in vitro links between MSCs and pericytes, identifying a population of CD146+CD34−CD45−CD56− cells as pericytes in several tissues [22]. These cells also expressed the classic MSC markers CD44, CD73, CD90, and CD105 in vivo but did not express endothelial markers CD31, CD144, vWF, or UEA-1. Crisan et al. concluded that cultured perivascular cells from a variety of tissues exhibit a phenotype that is very similar to that of BM-MSCs [22]. Because of their role as pericytes in most tissues, MSCs were now suggested to have an important role in vasculogenesis by stabilizing the vasculature [22, 23, 40–42]. Through interaction with ECs [23], the MSCs are thus able to stimulate angiogenesis [35, 43]. In conclusion, most evidence today suggests that the main role of AT-MSCs in blood vessel biology may be as pericytes to secrete angiogenic factors and stabilize the interactions between ECs.

### Endothelial Cells in Adipose Tissue

Based on cell surface expression of CD31 and HLA DR, intracellular expression of vWF, and very high expression of mRNAs typical of ECs, the CD31− subset of SVF cells was considered to consist of ECs, most likely microvascular ECs [29]. Previously, several attempts had been made to isolate ECs from SVF using plastic attachment techniques and positive selection strategies [44–48]. We recently used a combination of negative and positive immunomagnetic isolation to derive a pure population of CD31− cells from AT [29]. Depending on the amount of liposuction starting material, several tens of millions of uncultured ECs could be obtained. These cells were readily expandable on a gelatin coat with an endothelial culture medium supplemented with fetal bovine serum (FBS). Later, we replaced the FBS with human plasma supplemented with human platelet lysate (PLP). This has enabled us to culture the ECs directly on plastic surfaces, which means that the culture system is entirely humanized. These AT-ECs proliferate rapidly through at least 15–20 population doublings. Since the starting number of cells is already high, several hundred millions of ECs may be obtained after a relatively brief period of in vitro expansion. The AT-ECs form functional blood vessels in Matrigel following subcutaneous injection into immuno-deficient mice. Interestingly, the vessel formation was more dense and robust when AT-ECs were combined with the AT-MSCs, suggesting that the MSCs adopt a supportive role similar to that of pericytes under these conditions [29]. Nevertheless, the identity of the AT-ECs still remains controversial. Since they express CD144 (vascular endothelial cadherin) and vWF but do not express CD133, CD45, or CD14, they are not likely to represent a population of early EPCs [49, 50]. Expression of genetic markers typical of both the arterial and the venous side of capillaries suggests that they may be microvascular endothelial cells (MVECs). However, it has been shown that MVECs strongly express CD141 [51], which was only weakly expressed or absent on AT-ECs. Based on their phenotype (CD34+CD133−vWF+CD144+VEGFR2+endothelial nitric oxide synthase+CD31+), AT-ECs most resemble late outgrowth EPCs or ECFCs [49, 50]. A population of ECFCs isolated from peripheral blood has recently been described [20]. The isolation procedure was different from that used to isolate EPCs [13], and the authors suggested that these cells most closely resemble microvascular cells. In collaboration with this group, we are now performing studies comparing the blood-derived ECFCs and the AT-ECs in terms of gene expression, phenotype, and vessel-forming functionality. This study should also help to clarify the somewhat confusing terminology used for human ECs.

### Therapeutic Potential of Adipose Tissue-Derived Angiogenic Cells

AT, then, contains two populations of cells with different functionalities that may contribute to neovascularization: the bona fide ECs and AT-MSCs. These two nonoverlapping populations make up more than 60% of the SVF and may be isolated in large numbers from a relatively small amount of liposuction material. Some clinical studies are based on uncultured SVF [52]. The advantage is that isolation of SVF from liposuction material is a relatively rapid procedure, which in fact may be performed automatically in the operating room [53–55]. The disadvantages are the lower numbers of cells, a relatively uncontrolled mixture of cell populations, and the fact that the functionality of uncultured AT-ECs may be different from culture-expanded AT-ECs [29]. For isolation of pure populations of AT-MSCs and AT-ECs, negative immunomagnetic isolation procedures may be used for both [27, 29]. This leaves no immunomagnetic beads in the resulting cell population, a fact that should make the procedure acceptable for cells to be used for treatment of patients. Both
cell populations are readily expandable during in vitro culture, and both may be cultured using human PLP, which makes the entire ex vivo expansion procedure free of xenogeneic proteins. Thus, the ex vivo isolation and culture procedure is likely to be acceptable to national regulatory authorities.

The availability of autologous ECs is likely to be crucially important both for the tissue engineering of arteries and for cell therapy for microvascular disease. Most ECs express HLA class II antigens [56]. Uncultured AT-ECs express HLA II molecules but lose these quickly from the surface upon cell culture [29]. Human AT-MSCs cultured in FBS express HLA II at the mRNA level but not on the surface [27]. However, according to our recent observation, when human PLP is used as a supplement, some of the AT-MSCs express HLA class II antigens. Both of these cell populations are likely to upregulate HLA class II molecules in an inflammatory environment. Thus, allogeneic angiogenic cells are likely to be rejected by an allo-specific immune response directed toward their HLA class II molecules. The same may well be the fate for autologous angiogenic cells cultured in FBS, where xenogeneic antigens presented by autologous HLA class II molecules may induce an immune response. Autologous cells expanded in human medium supplements, however, are likely to be well tolerated in a transplantation situation.

However, there are still issues that need to be solved. One such issue is whether there are important phenotypic and functional differences in fat obtained from different sites [57]. The immunomodulatory property of AT-MSCs is also an important issue. It has been shown that AT-MSCs promote engraftment and prevent or treat severe graft-versus-host disease in allogeneic stem cell transplantation [58, 59]. Treatment with immunosuppressive cells might conceivably activate dormant infections or tumors, although results in this area are contradictory [60–63]. Also, in vitro culture of cells could activate transformation pathways and lead to tumor formation. MSCs are known to occasionally form tumors in mice [64], but neither tumors nor ectopic tissue formation following injections of MSCs in humans has been reported after more than 10 years of follow-up [64–66]. Clinical application of AT-MSCs may therefore be considered to be safe. The preclinical and clinical experience with cultured ECs is still limited [67]. Thus, additional studies are needed to fully elucidate the safety and reproducibility of the in vitro expanded ECs.

Based on the ready availability of large numbers of autologous cells, AT-ECs are likely to be attractive EC candidates for scientists involved in tissue engineering of arterial vessels. However, very few clinical trials using cell-based approaches to tissue engineer blood vessels have so far been performed [68]. In contrast, a huge number of clinical trials of stem cell therapy have been performed in attempts to moderate the outcome of another arterial disease, acute myocardial infarction (AMI) [69, 70]. Most of these have used uncultured populations of autologous cells derived from the bone marrow. The results of these trials are so far that “stem/progenitor cell treatment was not associated with statistically significant changes in the incidence of mortality [relative risk] 0.70, 95% CI 0.40–1.21) or morbidity (the latter measured by reinfarction, hospital readmission, restenosis and target vessel revascularization)” [69]. It is possible that the treatment outcome could have been improved by injection of autologous cells with angiogenic potential. However, AT-ECs and AT-MSCs need to be cultured in vitro to obtain the number of cells likely to induce neovascularization. This takes several weeks, by which time the acute phase of AMI has passed, and a therapeutic opportunity may have been lost. Cell culture-expanded autologous angiogenic cells from AT could be provided to patients in the acute phase of AMI, but as described above, these cells are likely to be rejected by alloimmune responses. Finally, relatively large numbers of uncultured autologous SVF cells may be procured within hours in an acute AMI situation. Human clinical trials using these cells are known to be under way, but no results have yet been published [52].

Ischemic symptoms caused by obstruction of arterioles and capillaries are not accessible to replacement or dilatation procedures and are currently treatable only by cell-based strategies for neovascularization. In the heart, this illness is called refractory angina pectoris. A number of clinical trials have been performed in groups of patients with refractory angina (Table 1). Most of these trials have used mononuclear cells from bone marrow (BM-MNCs). These are uncultured cells in which the fraction of hematopoietic stem cells is less than 1%, and the fraction of EPCs is less than that. However, some recent studies have used cell culture-expanded MSCs (Table 1). All of these studies have reported beneficial effects, some even after long observation periods. The mechanism of the beneficial effect is uncertain. In fact, cells injected into the heart usually do not remain there very long; they migrate to the lung, spleen, and other organs [71]. Those cells that remain in the heart usually die or do not function. There may be several explanations for this. To survive, cells need the appropriate signals from their environment. This is particularly important for cells expanded in vitro adherent to molecules on plastic surfaces. These environmental signals may not be available in the myocardium. Also, cells injected into ischemic myocardium may find the microenvironment too hostile to promote survival. Recently we injected several different populations of human MSCs into the border zones of 1-week-old myocardial infarctions in immunodeficient rats [72]. They all induced surprisingly good functional improvement. At 4 weeks, only a small fraction of the injected cells could be recovered in the murine myocardium. This study and all other studies reporting beneficial effects of cells injected into ischemic hearts suggest that the benefit is mediated by paracrine factors [73]. A priori, a combination of autologous AT-ECs and AT-MSCs injected intramyocardially in patients with refractory angina should do better than any of the cells injected in studies published to date (Table 1), because the potential for direct contribution to neovascularization is considerably greater for these cells. To achieve this, however, the problem of the survival of cultured cells injected into myocardium needs to be solved.

Also for critical limb ischemia that has not been amenable to dilatation procedures, a number of cell-based trials to establish neovascularization have been performed (Table 2). Again, the cells most commonly used have been BM-MNCs, and clinical improvement has also been recorded in these groups of patients. Naturally, direct proof of involvement of the injected cells in the establishment of new blood vessels is not available, but in several of these studies evidence of improved blood supply to the ischemic regions could be demonstrated. In a mouse model of hind limb ischemia, evidence supporting the survival and direct contribution to new blood vessel formation by injected human AT-MSCs has been published [35–37]. This would suggest that the likelihood of survival of injected angiogenic cells in limb tissues is better than in the myocardium. If so, injection of combinations of autologous AT-ECs and AT-MSCs is likely to give an
Table 1. Clinical trials of cell-based therapy in therapeutic angiogenesis for refractory angina

| Clinical study          | Type and no. of cells                                                                 | Delivery | No. of patients/follow-up | Results                                                                                   |
|-------------------------|--------------------------------------------------------------------------------------|----------|---------------------------|-------------------------------------------------------------------------------------------|
| Fuchs et al. [74]       | Unfractionated BM cells (32.6 ± 27.5 × 10^6/ml nucleated cells containing 2.6 ± 1.6% CD34<sup>+</sup>) | IM       | 10 patients/3 months      | Improved CCSAC, exercise duration, stress-induced ischemia score                           |
| Vicario et al. [75]     | Unfractionated BM cells (0.089 ± 0.023 × 10^6/kg nucleated cells)                     | IC       | 15 patients/12 months     | Improved quality of life, CCSAC, myocardial perfusion                                     |
| Fuchs et al. [76]       | Unfractionated BM cells (28 ± 27 × 10^6/ml nucleated cells containing 2.2 ± 1.4% CD34<sup>+</sup>) | IM       | 27 patients/3 and 12 months | Improved CCSAC, exercise duration, stress-induced ischemia score                           |
| Beeres et al. [77]      | BM-MNCs (84 ± 29 × 10<sup>6</sup>)                                                     | IM       | 25 patients/12 months     | Improved CCSAC, quality of life, LVEF, regional wall motion; reduced ischemic area        |
| Tse et al. [78]         | BM-MNCs (10<sup>6</sup>/injection)                                                    | IM       | 12 patients/44 ± 10 months| No change in LVEF; most of the patients developed major cardiovascular events              |
| Briguori et al. [79]    | BM-MNCs (10<sup>7</sup>/injection)                                                    | IM       | 10 patients/12 months     | Improved CCSAC, myocardial perfusion, quality of life                                     |
| Boyle et al. [80]       | Mobilized CD34<sup>+</sup> (66.9 ± 17.6 × 10<sup>5</sup>)                            | IC-XRF   | 5 patients/12 months      | Improved CCSAC, quality of life                                                           |
| Tse et al. [81]         | BM-MNCs (1 × 10<sup>6</sup>/2 × 10<sup>6</sup> per 0.1 ml)                           | IM       | 28 patients/6 months      | Improved exercise tolerance, CCSAC, NYHA class, LVEF                                     |
| Losordo et al. [82]     | Mobilized CD34<sup>+</sup> (5 × 10<sup>5</sup>, 1 × 10<sup>6</sup>, or 5 × 10<sup>5</sup> cells/kg) | IM       | 24 patients/12 months     | Improved angina frequency, CCSAC, exercise tolerance                                      |
| Pompilio et al. [83]    | CD133<sup>+</sup> BMSCs (4–12 × 10<sup>5</sup>)                                     | IM       | 5 patients/12 months      | Improved CCSAC, myocardial perfusion; increase in collateral score                        |
| van Ramshorst et al. [84]| BM-MNCs (100 × 10<sup>6</sup>)                                                        | IM       | 50 patients/6 months      | Improved CCSAC, LVEF, quality of life, summed stress score                                |
| Hossne et al. [85]      | BM-MNCs (2 × 10<sup>6</sup>/injection)                                               | IM       | 8 patients/18 months      | Improved CCSAC; reduced ischemic area                                                     |
| Wang et al. [86]        | BM-CD34<sup>+</sup> (5.6 ± 2.3 × 10<sup>7</sup>)                                     | IC       | 112 patients/6 months     | Improved CCSAC, myocardial perfusion, exercise capacity; reduced angina frequency episodes|
| Lasala et al. [87]      | BM-MSCs + BM-MNCs (7.5 × 10<sup>5</sup>/population)                                  | IC       | 10 patients/6 months      | Improved LVEF, quality of life                                                           |
| Friis et al. [88]       | BM-MSCs derived EPCs (21.5 × 10<sup>6</sup>)                                        | IM       | 31 patients/6 months      | Improved LVEF, exercise capacity, clinical symptoms                                       |
| Losordo et al. [89]     | Mobilized CD34<sup>+</sup> (1 × 10<sup>5</sup> or 5 × 10<sup>5</sup> cells/kg)        | IM       | 167 patients/12 months    | Improved angina frequency, exercise tolerance                                             |
| Tuma et al. [90]        | BM-MNCs (8.19 ± 4.3 × 10<sup>6</sup> + CD34<sup>+</sup> (1.65 ± 1.42 × 10<sup>7</sup>) | PRCSP   | 14 patients/24 months     | Improved CCSAC, LVEF; reduced ischemic area                                              |
| Haack-Sørensen et al. [91]| BM-MSCs (21.5 × 10<sup>6</sup>)                                                       | IM       | 31 patients/12 months     | Improved CCSAC, nitroglycerine consumption, physical limitation, angina frequency, quality of life |

Abbreviations: BM, bone marrow; BM-MNC, bone marrow mononuclear cell; BM-MSC, bone marrow mesenchymal stem cell; BMSMC, bone marrow stem cell; CCSAC, Canadian Cardiovascular Society Angina Classification; EPC, endothelial progenitor cell; IC, intracoronary infusion; IC-XRF, intracoronary-x-ray fluoroscopy; IM, intramyocardial injection; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PRCSP, percutaneous retrograde coronary sinus perfusion.

Even better clinical outcome than those reported in the studies cited in Table 2.

**Conclusion**

Traditional risk factors such as smoking, diabetes, hypercholesterolemia, hypertension, and age itself can lead to endothelial injury requiring repair of the vasculature. Surgical and catheter-based procedures are constantly improving the treatment options for many patients with tissue ischemia, but diseases mainly affecting arterioles and capillaries are likely to never be amenable to surgical or dilatation procedures. For these, cell-based therapeutic strategies will remain the best treatment options.

Two populations of cells with different functionalities exist in the SVF of AT that may contribute to neovascularization. Both may be used in the uncultured state, when the cell numbers will be lower but the cells are quickly available, and after in vitro expansion. Combinations of in vitro expanded AT-ECs and AT-MSCs yield robust vasculogenesis in Matrigel plugs injected into immunodeficient rodents, suggesting that these cells might also provide relief from ischemia in human clinical situations. However, several issues have to be addressed in order to get full therapeutic benefit from these cells. Most importantly, the cells must be seen to survive and directly contribute to new blood vessel formation. Another important issue is the mode of administration: should the cells be injected into the arterial tree supplying the ischemic area, or into the tissue actually exposed to ischemia? In addition, the role of supportive angiogenic cytokines and growth factors such as VEGF, FGF, HGF, and angiopoietin-1 remains unresolved. If one or more of these should be found to be beneficial, a decision has to be made whether the cytokines should be provided by supplemental injection or by genetically manipulating the injected cells.

The success of cell-based therapies depends on whether the engrafted cells differentiate into functional vascular cells and whether those cells can produce paracrine signals that encourage survival of the cells in the ischemic environment. Animal studies will be required to understand induced vasculogenesis in
Table 2. Clinical trials of cell-based therapy in therapeutic angiogenesis for critical limb/hand ischemia

| Clinical study | Type and no. of cells | Delivery | No. of patients/ follow-up | Results |
|----------------|-----------------------|----------|-----------------------------|---------|
| Lenk et al. [92] | CPCs (CD34\(^+\)CD144\(^-\)) (39 ± 24 x 10\(^6\)) | IA | 7 patients /12 weeks | Improved pain-free walking distance, ABI, tissue blood perfusion |
| Huang et al. [93] | Mobilized PB-MNCs (3 x 10\(^9\)) | IM | 28 patients /3 months | Less pain; smaller ulcer size; improved tissue blood perfusion and ABI; increased limb salvage |
| Koshikawa et al. [94] | BM-MNCs (3.67 ± 0.53 x 10\(^7\)), CD34\(^+\) (4.94 ± 2.45 x 10\(^7\)), CD34\(^-\)CD133\(^+\) (2.52 ± 1.57 x 10\(^7\)) | IM | 7 patients /6 months | Improved perfusion and ulcer size; less pain |
| Bartsch et al. [95] | BM-MNCs (8.3 ± 34 x 10\(^5\)) | IM and IA | 13 patients /13 months | Less pain; disappearance of gangrene; neovascularization |
| Hernández et al. [96] | BM-MNCs (1.71 ± 1.23 x 10\(^7\)), CD34\(^+\) (8.14 ± 6.67 x 10\(^7\))7.9 ± 5.46 x 10\(^7\)) | IM | 12 patients /24 weeks | Improved ABI, rest pain, and pain-free walking time |
| Kajiguchi et al. [97] | BM-MNCs/PB-MNCs (4 x 10\(^6\) to 7 x 10\(^7\)) | IM | 7 patients /23.7 months | Less pain; improved tissue blood perfusion; unchanged ABI |
| Matoba et al. [98] | BM-MNCs | IM | 115 patients /25.3 months | Improved ulcer size, pain scale, pain-free walking distance, and tissue blood perfusion |
| van Tongeren et al. [99] | BMCs (1.23 ± 0.49 x 10\(^5\))CD34\(^+\) (3.07 ± 2.02 x 10\(^5\)) | IM vs. IM + IA | 27 patients /24 ± 8 months | Increased limb salvage; improved pain-free walking distance and ABI; less pain |
| Wester et al. [100] | BM-MNCs (1.3 x 10\(^5\)) | IM | 8 patients /8 months | Pain relief |
| Chochola et al. [101] | BM-MNCs CD34\(^+\) (34.9 ± 10\(^6\)) | IA | 24 patients /1 year | Increased limb salvage; improved ulcer healing and collateral vessel development |
| Cobellis et al. [102] | BMCS (10\(^7\)) | IA | 10 patients /12 months | Improved tissue blood perfusion, ABI, capillary densities, and pain-free walking distance |
| Napoli et al. [103] | BMCs + oral antioxidants, l-arginine therapy (5 x 10\(^7\)/ml) | IA | 36 patients /18 months | Improved pain-free walking distance, ABI, and ulcer healing |
| Kawamoto et al. [104] | Mobilized CD34\(^+\) (10\(^5\) or 5 x 10\(^5\) or 10\(^6\) cells/kg) | IM | 17 patients /12 weeks | Improved ulcer size, exercise capacity, and transcutaneous partial oxygen pressure; less pain |
| Amann et al. [105] | BM-MNCs (1.1 ± 1.1 x 10\(^3\))/BM total nucleated cells (3 ± 1.7 x 10\(^5\)) | IM | 51 patients /6 months | Increased limb salvage; improved ABI, pain-free walking distance, and tissue blood perfusion |
| Burt et al. [106] | CD133\(^+\) (2.5–5 x 10\(^5\)/injection) | IM | 9 patients /12 months | Improved amputation-free survival, quality of life, exercise capacity, perfusion, and collateral formation; less pain |
| Lasala et al. [107] | BM-MNCs (30 x 10\(^5\)) with BM-MSCs (30 x 10\(^5\)) | IA | 10 patients /10 ± 2 months | Improved amputation-free walking distance, and cumulative survival |
| Kolvenbach et al. [108] | BMCs (17.2 ± 10\(^6\)CD34\(^+\); 7.8 ± 10\(^6\)CD133\(^+\); 0.5–5.7 x 10\(^5\)VEGFR2\(^+\)) | IM | 8 patients /9.2 months | Improved ABI |
| Lara-Hernandez et al. [109] | Mobilized EPCs (CD34\(^+\)CD133\(^-\)) | IM | 28 patients /14 months | Improved ABI; less pain; increased limb salvage |
| Sprengers et al. [110] | EPCs/BM-MNCs | IA | 110–160 patients | Ongoing |
| Procházková et al. [111] | BM-MSCs | IM | 96 patients /4 months | Improved limb salvage |
| Walter et al. [112] | BM-MNCs (87 ± 29 x 10\(^6\) or 178 ± 113 x 10\(^6\)) | IA | 40 patients /30.2 months | Improved ulcer healing; reduced rest pain; negative limb perfusion |
| Powell et al. [113] | BM-MNCs (136 ± 41 x 10\(^6\)) | IM | 46 patients /6 months | Improved amputation-free survival and ulcer healing |
| Idei et al. [114] | BM-MNCs (1.8 ± 0.5 x 10\(^6\)), CD34\(^+\) (3.5 ± 1.4 x 10\(^7\)) | IM | 97 patients /4.8 years | Improved amputation-free survival and cumulative survival |
| Lu et al. [115] | BM-MSCs/BM-MNCs | IM | 41 patients /24 weeks | Improved pain-free walking time, ulcer healing, tissue blood perfusion, and ABI |
| Murphy et al. [116] | BM-MNCs (1.7 ± 0.7 x 10\(^9\)) | IM | 29 patients /1 year | Improved ABI, amputation-free survival, perfusion index, ulcer healing, and quality of life |
| Perin et al. [117] | BM-MNCs vs. aldehyde dehydrogenase bright BM-MNCs (1.3 ± 1 x 10\(^5\) vs. 1.36 ± 0.59 x 10\(^5\)) | IM | 21 patients /12 weeks | Improved ABI; unchanged ulcer grade |
| Gabr et al. [118] | BM-MNCs (1.11 ± 10\(^5\)) | IM | 20 patients /3 months | Improved pain-free walking distance, resting pain, skin conditions, and ABI |

Abbreviations: ABI, ankle brachial index; BMC, bone marrow cell; BM-MNC, bone marrow mononuclear cell; BM-MSC, bone marrow mesenchymal stem cell; CLI, critical limb ischemia; CPC, circulating blood-derived progenitor cell; EPC, endothelial progenitor cell; IA, intra-arterial injection; IM, intramuscular injection; PB-MNC, peripheral-blood mononuclear cell; VEGFR, vascular endothelial growth factor receptor.
the suboptimal ischemic vascular environment and to ensure that treatment with angiogenic cells is safe. Issues such as nurturing the local environment and appropriate delivery methods are key issues that need to be resolved before successful regenerative therapies will be effective in patients.

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REFERENCES

1. Weber C, Noels H. Atherosclerosis: Current pathogenesis and therapeutic options. Nat Med 2011;17:1410–1422.
2. Neurem RM, Seliktar D. Vascular tissue engineering. Annu Rev Biomed Eng 2001;3:225–243.
3. Eltzschig HK, Eckle T. Ischaemia and reperfusion—from mechanism to translation. Nat Med 2011;17:1391–1401.
4. Collinson DJ, Donnelly R. Therapeutic angiogenesis in peripheral arterial disease: Can biotechnology produce an effective collateral circulation? Eur J Vasc Endovasc Surg 2004;28:9–23.
5. Carmeliet P. Angiogenesis in health and disease. Nat Med 2003;9:653–660.
6. Freedman SB, Isner JM. Therapeutic angiogenesis for ischemic cardiovascular disease. J Mol Cell Cardiol 2001;33:379–393.
7. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: Role of the HIF system. Nat Med 2003;9:677–684.
8. Folkman J, D’Amore PA. Blood vessel formation: What is its molecular basis? Cell 1996;87:1153–1155.
9. Drake CJ. Embryonic and adult vasculogenesis. Birth Defects Res C Embryo Today 2003;69:73–82.
10. Eguchi M, Masuda H, Asahara T. Endothelial progenitor cells for postnatal vasculogenesis. Clin Exp Nephrol 2007;11:18–25.
11. Adamo L, Garcia-Cardena G. The vascular origin of hematopoietic cells. Dev Biol 2012;362:1–10.
12. 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.
13. Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–967.
14. Fadini GP, Losordo D, Dimmel S. Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. Circ Res 2012;110:624–637.
15. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vasculogenesis and regeneration. Nat Med 2003;9:702–712.
16. 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.

17. Takahashi T, Kalka C, Masuda H et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434–438.
18. Prater DN, Case J, Ingram DA et al. Working hypothesis to redefine endothelial progenitor cells. Leukemia 2007;21:1141–1149.
19. Huy J, Yoon CH, Kim HS et al. Characterization of two types of endothelial progenitor cells and their different contributions to neovascularogenesis. Artroscler Thromb Vasc Biol 2004;24:288–293.
20. Reinsch A, Hofmann NA, Obenauf A et al. Humanized large-scale expanded endothelial colony-forming cells function in vitro and in vivo. Blood 2009;113:6716–6725.
21. Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. Stem Cell Rev 2011;7:269–291.
22. Crisan M, Yap S, Castella L et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301–313.
23. Traktuev DO, Merfeld-Claus S, Li J et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 2008;102:77–85.
24. Cao Y. Angiogenesis modulates adipogenesis and obesity. J Clin Invest 2007;117:2362–2368.
25. Zuk PA, Zhu M, Mizuno H et al. Multilineage human cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng 2001;7:211–221.
26. Zuk PA, Zhu M, Ashjian P et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–4295.
27. Boquest AC, Shahadaddar A, Fronsdal K et al. Isolation and transcription profiling of purified uncultured human stromal stem cells: Alteration of gene expression after in vitro cell culture. Mol Biol Cell 2005;16:1131–1141.
28. Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.
29. Szkòe K, Beckstrom KJ, Brinchmann JN. Human adipose tissue as a source of cells with angiogenic potential. Cell Transplant 2012;21:235–250.
30. Fraser JK, Wulur I, Alfonso Z et al. Fat tissue: an unappreciated source of stem cells for biotechnology. Trends Biotechnol 2006;24:150–154.
31. Ouma GO, Jonas RA, Usman MH et al. Targets and delivery methods for therapeutic angiogenesis in peripheral artery disease. Vasc Med 2011;16:171–179.
32. Rehman J, Traktuev D, Li J et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004;109:1292–1298.
33. Nakagami H, Maeda K, Morishita R et al. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. Artroscler Thromb Vasc Biol 2005;25:2542–2547.
34. Reichenberger MA, Heimer S, Schaefer A et al. Adipose derived stem cells protect skin flaps against ischemia-reperfusion injury. Stem Cell Rev 2012;8:854–862.
35. Miranville A, Heeschen C, Sengenes C et al. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 2004;110:349–355.
36. Planat-Benard V, Silvestre JS, Cousin B et al. Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. Circulation 2004;109:656–663.
37. Moon MH, Kim SY, Kim YJ et al. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. Cell Physiol Biochem 2006;17:197–209.
38. Kondo K, Shintani S, Shibata R et al. Implantation of adipose-derived regenerative cells enhances ischemia-induced angiogenesis. Artroscler Thromb Vasc Biol 2009;29:61–66.
39. Covas DT, Panepucci RA, Fontes AM et al. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profiles with CD146+ perivascular cells and fibroblasts. Exp Hematol 2008;36:642–654.
40. da Silva ML, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. Stem Cells 2008;26:2287–2299.
41. Caplan AI. All MSCs are pericytes? Cell Stem Cell 2008;3:229–230.
42. Corselli M, Chen CW, Crisan M et al. Perivascular ancestors of adult multipotent stem cells. Artroscler Thromb Vasc Biol 2010;30:1104–1109.
43. von Tell D, Armulik A, Betsholtz C. Pericytes and vascular stability. Exp Cell Res 2006;312:623–629.
44. Arts CH, de GP, Heijnen-Snyder GJ et al. Application of a clinical grade CD34-mediated method for the enrichment of microvascular endothelial cells from fat tissue. Cytotherapy 2006;8:30–42.

AUTHOR CONTRIBUTIONS

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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favor tumor cell growth in vivo. Stem Cells Dev 2008;17:463–473.
17. Muehlberg FI, Song YH, Krohn A et al. Tissue-resident stem cells promote breast cancer growth and metastasis. Carcinogenesis 2009;30:589–597.
18. Cousin B, Ravet E, Foglio S et al. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. PLoS One 2009;4:e6278.
19. Casiraghi F, Remuzzi G, Abbate M et al. Multipotent mesenchymal stromal cell therapy and risk of malignancies. Stem Cell Rev 2012 [Epub ahead of print].
20. von Bahr L, Batts I, Moll G et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. Stem Cells 2012;30:1575–1578.
21. Lee JS, Hong JM, Moon GI et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells 2010;28:1099–1106.
22. Kaneko Y, Tajiri N, Shinozuka K et al. Cell therapy for stroke: Focus on optimizing safety and efficacy profile of endothelial progenitor cells. Curr Pharm Des 2012;18:3731–3734.
23. Peck M, Gebhart D, Dusserre N et al. The evolution of vascular tissue engineering and current state of the art. Cells Tissues Organs 2012;195:144–158.
24. Clifford DM, Fisher SA, Brunkuss SJ et al. Stem cell treatment for acute myocardial infarction. Cochrane Database Syst Rev 2012:2:CD006536.
25. Beitnes JO, Lunde K, Brinchmann JE et al. Stem cell therapy for acute myocardial infarction. Expert Rev Cardiovasc Ther 2011;9:1015–1025.
26. Robich MP, Chu LM, Oyamada S et al. Myocardial therapeutic angiogenesis: A review of the state of development and future obstacles. Expert Rev Cardiovasc Ther 2011;9:1469–1479.
27. Beitnes JO, Oie E, Shahdadfar A et al. Intramyocardial injections of human mesenchymal stem cells following acute myocardial infarction modulate scar formation and improve left ventricular function. Cell Transplant 2012 [Epub ahead of print].
28. Wollert KC, Drexler H. Cell therapy for the treatment of coronary heart disease: A critical appraisal. Nat Rev Cardiol 2010;7:204–215.
29. Fuchs S, Satler LF, Kornowski R et al. Catheter-based autologous bone marrow myocardial injection in no-opinion patients with advanced coronary artery disease: A feasibility study. J Am Coll Cardiol 2003;41:1721–1724.
30. Vicario J, Campo C, Piva J et al. One-year follow-up of transcoronary sinus administration of autologous bone marrow in patients with chronic refractory angina. Cardiovasc Revasc Med 2005;6:99–107.
31. Fuchs S, Kornowski R, Weisz G et al. Safety and feasibility of transcatheter autologous bone marrow cell transplantation in patients with advanced heart disease. Am J Cardiol 2006;97:823–829.
32. Beeser SL, Bax JJ, Dibbets-Schneider P et al. Sustained effect of autologous bone marrow mononuclear cell injection in patients with refractory angina pectoris and chronic myocardial ischemia: Twelve-month follow-up results. Am Heart J 2006;152:684.e11–684.e16.
33. Tse HF, Thambir S, Kwong YL et al. Safety of catheter-based intramyocardial autologous bone marrow cells implantation for therapeutic angiogenesis. Am J Cardiol 2006;98:60–62.
34. Briggs G, Reimers B, Sarais C et al. Direct intramyocardial percutaneous delivery of autologous bone marrow in patients with refractory myocardial angina. Am Heart J 2006;151:674–680.
35. Boyle AJ, Whitbourn R, Schlicht S et al. Intra-coronary high-dose CD34 stem cells in patients with chronic ischemic heart disease: A 12-month follow-up. Int J Cardiol 2006;109:21–27.
36. Tse HF, Thambir S, Kwong YL et al. Prospective randomized trial of direct endomyocardial implantation of bone marrow cells for treatment of severe coronary artery diseases (PROTECT-CAD trial). Eur Heart J 2007;28:2998–3005.
37. Losordo DW, Schatz RA, White CJ et al. Intramyocardial transplantation of autologous CD34 stem cells for intractable angina: A phase I/IIa double-blind, randomized controlled trial. Circulation 2007;115:3165–3172.
38. Pompilio G, Steinhoff G, Liebold A et al. Direct minimally invasive intramyocardial injection of bone marrow-derived AC133 stem cells in patients with refractory ischemia: Preliminary results. Thorac Cardiovasc Surg 2008;56:71–76.
39. van Ramshorst J, Bax JJ, Beeser SL et al. Intramyocardial bone marrow cell injection for chronic myocardial ischemia: A randomized controlled trial. JAMA 2009;301:1997–2004.
40. Hosse NA, Jr., Invitti AL, Buffolo E et al. Refractory angina cell therapy (ReACT) involving autologous bone marrow cells in patients without left ventricular dysfunction: A possible role for monocytes. Cell Transplant 2009;18:1299–1310.
41. Wang S, Cui J, Peng W et al. Intracoronary autologous CD34 stem cell therapy for intractable angina. Cardiology 2010;117:140–147.
42. Lasala GP, Silva JA, Kusnacka B et al. Combination stem cell therapy for the treatment of medically refractory coronary ischemia: A Phase I study. Cardiovasc Revasc Med 2011;12:29–34.
43. Friis T, Haack-Sørensen M, Mathiasen AB et al. Mesenchymal stromal cell derived endothelial progenitor therapy in patients with refractory ischemia. Scand Cardiovasc J 2011;45:161–168.
44. Losordo DW, Henry TD, Davidson C et al. Intramyocardial, autologous CD34 cell therapy for refractory angina. Circ Res 2011:109:428–436.
45. Tuma J, Fernandez-Vina R, Carrasco A et al. Safety and feasibility of percutaneous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina. J Transl Med 2011;9:183.
46. Haack-Sørensen M, Friis T, Mathiasen AB et al. Direct intramyocardial mesenchymal stromal cell injections in patients with severe refractory angina: One year follow-up. Cell Transplant 2012 [Epub ahead of print].
Rationale and design of the JUVENTAS trial for repeated intra-arterial infusion of autologous bone marrow-derived mononuclear cells in patients with chronic limb ischemia. J Vasc Surg 2011;53:1565–1574.

Perin EC, Silva G, Gahremanpour A et al. A randomized, controlled study of autologous therapy with bone marrow-derived aldehyde dehydrogenase bright cells in patients with critical limb ischemia. Catheter Cardiovasc Interv 2011;78:1060–1067.

Gabr H, Hedayet A, Imam U et al. Limb salvage using intramuscular injection of unfractionated autologous bone marrow mononuclear cells in critical limb ischemia: A prospective pilot clinical trial. Exp Clin Transplant 2011;9:197–202.

Bartsch T, Brehm M, Zeus T et al. Transplantation of autologous mononuclear bone marrow stem cells in patients with peripheral arterial disease (the TAM-PAD study). Clin Res 2007;22:793–798.

Hernández P, Cortina L, Artaza H et al. Autologous bone-marrow mononuclear cell implantation in patients with severe lower limb ischemia: A comparison of using blood cell separator and Ficoll density gradient centrifugation. Atherosclerosis 2007;194:e52–e56.

Kajiguichi M, Kondo T, Izawa H et al. Safety and efficacy of autologous progenitor cell transplantation for therapeutic angiogenesis in patients with critical limb ischemia. Circ J 2007;71:196–201.

Matoba S, Tatsumi T, Murohara T et al. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. Am Heart J 2008;156:1010–1018.

Van Tongeren RB, Hamming JF, Fibbe WE et al. Intramuscular or combined intramuscular/intra-arterial administration of bone marrow mononuclear cells: A clinical trial in patients with advanced limb ischemia. J Cardiovasc Surg (Torino) 2008;49:51–58.

Wester T, Jorgensen JJ, Stranden E et al. Treatment with autologous bone marrow mononuclear cells in patients with critical lower limb ischaemia. A pilot study. Scand J Surg 2008;97:56–62.

Chochola M, Pytlík R, Kobylka P et al. Autologous intra-arterial infusion of bone marrow mononuclear cells in patients with critical limb ischemia. Int Angiol 2008;27:281–290.

Cobellis G, Silvestroni A, Lillo S et al. Long-term effects of repeated autologous transplantation of bone marrow cells in patients affected by peripheral arterial disease. Bone Marrow Transplant 2008;42:667–672.

Napoli C, Farzati B, Sica V et al. Beneficial effects of autologous bone marrow cell infusion and antioxidants/-arginine in patients with chronic critical limb ischemia. Eur J Cardiovasc Prev Rehabil 2008;15:709–718.

Kawamoto A, Katayama M, Handa N et al. Intramuscular transplantation of G-CSF-mobilized CD34(+) cells in patients with critical limb ischemia: A phase I/IIa, multicenter, single-blinded, dose-escalation clinical trial. Stem Cells 2009;27:2857–2864.

Amann B, Luedemann C, Ratei R et al. Autologous bone marrow cell transplantation increases leg perfusion and reduces amputations in patients with advanced critical limb ischemia due to peripheral artery disease. Cell Transplant 2009;18:371–380.

Burt RK, Testori A, Oyama Y et al. Autologous peripheral blood CD133+/CD34+ cells for limb salvage in patients with critical limb ischemia. Bone Marrow Transplant 2010;45:111–116.

Lasala GP, Silva JA, Gardner PA et al. Combination stem cell therapy for the treatment of severe limb ischemia: Safety and efficacy analysis. Angiology 2010;61:551–556.

Kolvenbach R, Kreissig C, Cagianos C et al. Intraoperative adjunctive stem cell treatment in patients with critical limb ischemia using a novel point-of-care device. Ann Vasc Surg 2010;24:367–372.

Lara-Hernandez R, Lozano-Vilardell P, Blanes P et al. Safety and efficacy of therapeutic angiogenesis as a novel treatment in patients with critical limb ischemia. Ann Vasc Surg 2010;24:287–294.

Sprengers RW, Moll FL, Teraa M et al. Rationale and design of the JUVENTAS trial for repeated intra-arterial infusion of autologous bone marrow-derived mononuclear cells in patients with critical limb ischemia. J Vasc Surg 2010;51:1564–1568.

Procházka V, Gumulec J, Jalukva F et al. Cell therapy, a new standard in management of chronic critical limb ischemia and foot ulcer. Cell Transplant 2010;19:1413–1424.

Walter DH, Kranehnberg H, Balzer JO et al. 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.

Powell RJ, Comerota AJ, Berceli SA et al. Interim analysis results from the RESTORE-CLI, a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells and placebo in patients with critical limb ischemia. J Vasc Surg 2011;54:1032–1041.

Idei N, Soga J, Hata T et al. Autologous bone marrow mononuclear cell implantation reduces long-term major amputation risk in patients with critical limb ischemia: A comparison of atherosclerotic peripheral arterial disease and Buerger disease. Circ Cardiovasc Interv 2011;4:15–25.

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.

Murphy MP, Lawson JH, Rapp BM et al. Autologous bone marrow mononuclear cell therapy is safe and promotes amputation-free survival in patients with critical limb ischemia. J Vasc Surg 2011;53:1565–1574.

Gabr H, Hedayet A, Imam U et al. Limb salvage using intramuscular injection of unfractionated autologous bone marrow mononuclear cells in critical limb ischemia: A prospective pilot clinical trial. Exp Clin Transplant 2011;9:197–202.