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# Stem Cell Transplantation for the Treatment of Peripheral Arterial Disease

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## 1. Introduction

Peripheral Arterial Disease (PAD) is a chronic occlusion of lower-extremity arteries distal to the aortic bifurcation [1, 2]. It affects 3%-12% in the general population and its frequency increases with age [1-3]. In the vast majority of patients, PAD is ascribable to atherosclerosis [1,2 ]. It is a progressive disease leading to severe haemodynamic compromise of the affected extremity and may be even limb threatening in the event of critical ischaemia [1-3]. Not only does PAD have a considerable unfavourable impact on the quality of life, but it also poses a tremendous economic burden on society [1-3] and is an ominous harbinger of high morbidity and mortality due to concomitant coronary artery disease and cerebrovascular disease [4-6].

Management of PAD may be divided into medical and surgical. The former includes risk factor modification and medical treatment [3, 5, 7, 8], while the latter comprises surgical revascularisation (bypass surgery, intraluminal angioplasty, endovascular surgery) [9-11]. Revascularisation may be divided into percutaneous transluminal angioplasty (PTA) and by-pass surgery [9, 10, 12, 13]. The former can restore adequate blood flow, while recurrence is infrequent and amputation rates are rare [9-11]. This technique yields favourable results in patients with critical limb ischaemia as well [9, 10]. By-pass graft surgery has also been extensively practised [12, 13]. Depending on the location of affected arteries, it may be performed to the femoral, popliteal or even distal arteries, such as the dorsalis pedis artery [12, 13].

However, vascular atherosclerotic lesions may diffusely affect several anatomical regions. This holds especially true for elderly and diabetic patients, who may, therefore, be poor candidates for surgical intervention [1, 14]. Regrettably, it may also apply to patients with critical limb ischaemia or those who have had prior revascularisation [1, 15]. Hence, there is an undeniable need to develop alternative therapeutic modalities to restore limb blood flow [1, 5, 15, 16]. This chapter reviews the progress achieved with autologous stem cell transplantation, an important innovation involving intramuscular and/or intra-arterial stem cell administration into the affected lower extremity [15-17].

## 2. Stem cell therapy: Principles and cell types

Stem cell therapy is based on administration of autologous stem cells, taken either from the bone marrow, from the peripheral blood or, more rarely, from adipose tissue [17-23].

Human bone marrow contains stem cells with the potential for differentiation into a variety of tissues, including endothelium, liver, muscle, bone and skin [15-17, 22, 23]. Consequently, bone marrow-derived cells may differentiate into endothelial cells and also provide progenitor and/or stem cells to wounds during healing [15-17, 22, 23]. Importantly, the newly derived endothelial cells promote angiogenesis, i.e. new vessel formation (also called neovascularisation), by two mechanisms [15, 17, 24-26]. First, they get connected to each other and are organised into capillary tubes, thus forming the primary capillary plexus, which is further refined by remodelling [25, 26]. Secondly, they release growth factors promoting angiogenesis (also called angiogenic growth factors), mainly Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Hypoxia inducible factor-1 $\alpha$  (HIF-1  $\alpha$ ), Hepatocyte Growth Factor (HGF), Placental Growth Factor (PlGF), angiopoietin, Nerve Growth Factor (NGF), Developmental endothelial locus-1 (Del-1) and others [15, 24]. Similar efficacy appears to be also accomplished with peripheral blood stem cells and adipose tissue-derived stem cells [18-21, 23].

Bone marrow-derived cells include autologous bone marrow-derived bone marrow stem cells (BMCs), bone marrow mononuclear cells (BMMNCs) and endothelial progenitor cells (EPCs) [16, 18-20, 22, 23]. Peripheral blood-derived cells include mononuclear cells (PBMNCs), polymorphonuclear leukocytes (PMNCs), erythroid colony-forming cells (ECFCs), circulating blood-derived progenitor cells (CPCs) and EPC, while other cells mainly include mesenchymal stem cells (MSCs) and adipose tissue-derived cells (ADSCs) [16, 18-23].

### 3. Pre-clinical studies

Preclinical studies have evaluated a variety of stem cells from bone marrow, peripheral blood or other sources, using the ischaemic hindlimb model in the mouse, rat or rabbit. Experimental hindlimb ischaemia is achieved by surgical ligation of the superficial femoral and/or external iliac artery [15]. This unilateral hindlimb ischaemia has been widely used as a model of PAD, although it virtually equates to acute ischaemia, not entirely representative of chronic PAD [15]. Major studies are briefly described in this section.

#### 3.1 Bone marrow-derived stem cells

In the mouse ischaemic hindlimb, Ziegelhoeffer et al. [27] investigated whether BMCs act by incorporation into vessels. They found a threefold increase of such cells around growing collateral arteries in the ischaemic vs. control limb. The authors concluded that BMCs do not promote vascular growth by incorporating into vessel walls but may function as supporting cells [27]. In another study, intravenous BMC administration was successful in improving blood flow as well as increasing capillary density and expression of the proliferation-associated protein Ki-67 in mouse ischaemic hindlimbs [28]. Importantly, this beneficial effect was enhanced by concomitant metabolic vascular protection (1.0% vitamin E added to the chow and 0.05% vitamin C and 6% L-arginine added to the drinking water), suggesting an additive effect, attributable in part to NO activation and reduced systemic oxidative stress [28]. A further study provided evidence for increased neovascularisation with bone marrow-derived cells. Takagi et al. [29] examined whether granulocyte-colony stimulating factor (G-CSF) can enhance neovascularisation and collateral vessel formation induced by BMNC transplantation in the hindlimb ischaemia model. Administration of G-CSF significantly increased blood perfusion on Laser Doppler imaging (LDI), number of angiographically detectable collateral vessels, and capillary density [29]. Similarly, neovascularisation was

significantly increased in rats receiving BMNCs, as evidenced by angiographical findings and capillary density. The combination of G-CSF and BMNCs augmented neovascularisation in comparison to BMNCs alone, as manifested by angiographical findings and capillary density [29]. It was concluded that G-CSF and BMNCs combination could emerge as a helpful therapeutic approach to restore blood flow [29].

Sica et al. [30] explored the effect of intravenous BMC administration and concurrent metabolic vascular protection (1.0% vitamin E, 0.05% vitamin C, and 6% L-arginine) in the ischaemic hindlimb of diabetic and non-diabetic mice. In both mice, BMC treatment increased blood flow and capillary density and decreased interstitial fibrosis [30]. This effect was amplified by metabolic vasculoprotective treatment. The latter had by itself no effect on capillary density, but could reduce interstitial fibrosis in non-diabetic mice [30].

One year later, Jeon et al. [31] examined whether the angiogenic efficacy of a combination of two angiogenic strategies (EPC mobilization with G-CSF and BMMNC transplantation) is superior to either strategy alone in the mouse ischaemic hindlimb. Both treatments were efficacious in increasing microvessel density and expression of bFGF and VEGF [31]. Combination therapy accomplished more extensive expression of bFGF and VEGF than either form of single therapy [31].

More recently, a study randomised ApoE knockout mice to receive either phosphate buffered saline (PBS) or intra-arterial BMCs [32]. Efficacy was assessed by LDI of the ischaemic limbs. It was demonstrated that BMCs significantly ( $p < 0.05$ ) increased blood flow recovery in ischaemic limbs, in comparison to saline ( $61.8 \pm 15\%$  vs.  $41.9 \pm 13.9\%$ , respectively) [32]. Of note, it was revealed that BMCs differentiated not only into small blood vessels, but also into skeletal myofibres and supporting membranes [32]. The latter changes were associated with increased serum levels of VEGF, FGF-2, transforming growth factor beta (TGF $\beta$ ), interleukin 4 (IL-4), and tumour necrosis factor alpha (TNF- $\alpha$ ). These workers proposed that the benefit of new skeletal muscle formation might be added to that of angiogenesis in long-standing lower extremity ischaemia [32].

### 3.2 Stem cells from peripheral blood

Asahara et al. [33] showed increased capillary density and augmented blood flow recovery following administration of heterologous, homologous and autologous EPCs in the mouse ischaemic hindlimb. Athymic nude rats with unilateral hindlimb ischaemia, were randomly assigned to intramuscular administration of PBMNCs plus platelets or PBMNCs plus platelets plus PMNs [34]. Blood perfusion, as evaluated by LDI, improved significantly ( $p < 0.001$ ) by 44% in rats receiving PBMNC plus platelets. There was a further significant ( $p < 0.01$ ) increase with the addition of PMNs. Density of newly formed capillaries was increased by PBMNCs plus platelets (3.5-fold increase) or platelets alone (2.4-fold increase), with a significant between-group difference ( $p < 0.001$ ), whereas PMNs exerted a significant ( $p < 0.05$ ) inhibitory role (32% inhibition) [34].

Yoon et al. [35] utilised the synergistic effect of EPCs and outgrowth endothelial cells (OECs), both cultured from the peripheral blood. Each cell type alone succeeded in a significant ( $p < 0.05$ ) increase of new vessel formation in the mouse experimental model [35]. In vitro, there was a synergistic effect by mutual interaction through cytokines and matrix metalloproteinases (MMPs). In vivo, injection of both cell types resulted in significantly ( $p < 0.05$ ) more pronounced neovascularisation than either cell type alone [35].

In another study, rats were randomly allocated to MSCs, MNCs, or vehicle infusion (control group) [36]. Significant improvement of hindlimb ischemia was observed both in rats

receiving MNCs and in those treated with MSCs, as compared to the control group. LDI perfusion index was highest in the MSC group ( $0.81 \pm 0.08$ ), followed by the MNC ( $0.69 \pm 0.1$ ) and control group ( $0.57 \pm 0.06$ ) [36]. Capillary density was significantly ( $p < 0.01$ ) increased with both treatments compared to controls. MSC treatment was significantly ( $p < 0.01$ ) more efficacious in increasing capillary density than MNC treatment [36]. The number of transplanted cell-derived endothelial cells was also highest in MSC rats. Finally, MSCs were significantly ( $p < 0.05$ ) more tolerant to apoptotic stimuli (serum starvation and hypoxia) in vitro than MNCs [36].

Two further works confirmed the beneficial effect of EPCs from peripheral blood in hindlimb ischaemia of the mouse [37] and rat [38]. In the first study, EPCs improved histologically confirmed muscle healing, blood flow and vessel density [37]. These changes were associated with modulation of proangiogenic pathways: increased VEGF-A levels and sensitivity to VEGF family ligands, increased levels of monocyte chemotactic protein-1 (MCP-1) [37]. In the second work, EPC treatment was combined with targeted extracorporeal shock wave application to facilitate their tissue recruitment [38]. Combined treatment achieved a significant ( $p < 0.01$ ) increase in the number of histologically confirmed vascular endothelial growth factor-positive endothelial cells per myocyte, as well as a significant ( $p < 0.05$ ) enhancement in EPC recruitment and homing [38]. Moreover, there was a significant ( $p < 0.05$ ) increase in relative blood flow recovery assessed by LDI [38].

Using an immunodeficient hindlimb ischemia model and LDI, Sasaki et al. [39] evaluated limb salvage rate and blood perfusion after intramuscular implantation of ECFCs, as compared to PBS treatment in the controls. Salvage rate and blood perfusion were increased by 38% ( $p < 0.05$ ) and 82.8% ( $p < 0.01$ ), respectively [39]. Vascular smooth muscle cell recruitment was also increased and the capillary density was 1.6-fold higher ( $p < 0.05$ ) than in the control group [39]. Finally, ECFCs were confirmed to supply angiogenic cytokines (VEGF and FGF-2), suggesting a possible novel strategy for therapeutic angiogenesis [39].

### 3.3 Other stem cells

A variety of other cells have also been investigated. Niagara et al. [40] studied the effect of rabbit autologous primary skeletal myoblasts on angiographically confirmed new vessel formation. Treatment induced a significant increase in neovascularisation ( $p < 0.05$ ) and capillary density ( $p < 0.01$ ) [40].

Scientists from the Cardiovascular Research Institute in the Washington Hospital Center have experimented with MSCs in the mouse [41, 42]. They showed that these cells effectively increased VEGF and b-FGF levels, collateral blood flow and limb function [41, 42]. At the same time, auto-amputation and muscle atrophy were reduced. Their data indicated the great importance of paracrine signaling and, additionally, showed that cell incorporation into vessels was not a prerequisite for their effects [41, 42].

ADSCs represent another alternative. Cultured ADSCs from C57Bl/6 mouse inguinal adipose tissue were transplanted into the ischaemic mouse hindlimb and improved blood flow assessed by LDI and capillary density assessed by anti-CD31 immunostaining antibody [43]. The therapeutic effect appeared to be mainly achieved by their ability to secrete angiogenic growth factors: relatively high expression of HGF, VEGF, placental growth factor (PGF), and transforming growth factor beta (TGF)- $\beta$ , as well as moderate expression of FGF-2 and angiopoietin 1 were noted [43]. Moon et al. [21] have also shown that hADSC can improve blood flow (evidenced by LDI), even when transplanted relatively late, i.e. 7 days



post induction of ischaemia in mice. The therapeutic effect was related to the number of transplanted cells. Conditioned media from these cells increased proliferation of human aortic endothelial cells [21].

More recently, Kim and colleagues [44] have compared human adipose stromal cells (hADSC) with human bone marrow stromal cells (hBMSC) in a nude mice model of hindlimb ischaemia. The former showed superior recovery of blood flow and higher expression of matrix metalloproteinases (MMP3 and MMP9) than the latter, prompting further exploration of this therapeutic alternative [44].

## 4. Clinical studies

Clinical studies have evaluated stem cells taken from bone marrow, peripheral blood or other sources. These works differ substantially in number of patients recruited and endpoints used. Major studies are briefly described in this section (Tables 1, 2).

### 4.1 Bone marrow-derived stem cells

#### 4.1.1 BMCs

Esato et al. [45] administered BMCs in 8 selected patients with chronic lower extremity ischaemia (4 patients with PAD, 4 patients with thromboangiitis obliterans [TAO, B rger's disease]), in whom prior treatment had failed. Symptoms improved in 7/8 patients. Moreover, complete ulcer healing was achieved in 2/3 patients, and partial healing in the 3<sup>rd</sup> patient [45]. Temperature increase was documented in 2 patients and new collateral formation in 2 out of 3 patients who underwent angiography.

Nizankowski et al. [46] delivered BMCs intramuscularly by repeated injections into the pedal and tibial regions in 10 patients suffering from chronic leg ischaemia staged Fontaine IV. Efficacy was assessed by LDI, transcutaneous oxygen pressure (TcPO<sub>2</sub>), ankle-brachial index (ABI), visual analogue pain scale, analgesic therapy requirement, ulceration area, angiography and scintigraphy. Improved blood flow was documented by LDI and elevation in TcPO<sub>2</sub> [46]. Painful symptoms were relieved in the majority of patients, and only 3 amputations were finally needed at 12-month follow-up. Treatment efficacy did not depend on the number of cells injected [46].

Another centre recruited 22 patients, who were divided into those with severe and those with moderate ischaemia [47]. Both patient groups received BMCs, the number of which was higher in those with severe ischaemia. Evaluation included improvement of pain, cold sensation and numbness, ABI, TcPO<sub>2</sub>, angiography, amputation rate, and foot ulcer healing. At 4 weeks, pain was alleviated in 90.0% in patients with severe and 16.7% with moderate ischaemia ( $p < 0.01$ ) [47]. The corresponding rates of relief in cold sensation were 90.5% and 5.3% ( $p < 0.01$ ), while those in improved numbness were 62.5% and 9.1% ( $p < 0.01$ ) [47]. ABI was increased by 31.8% in subjects with severe ischaemia, but not in those with moderate ischaemia ( $p < 0.01$ ). The corresponding increments in TcPO<sub>2</sub> were 94.4% and 11.1% ( $p < 0.01$ ) [47]. Angiography demonstrated new collateral vessel network in 100% of subjects with severe ischaemia but not in the presence of moderate ischaemia ( $p < 0.01$ ) [47]. Subjects with severe ischaemia exhibited 4.5% amputation rates and 75% rates improved ulcer healing. The corresponding rates in those with moderate ischaemia were 27.3% and 0% ( $p < 0.05$ ). Thus, it was concluded that efficacy was significantly superior with higher number of implanted cells [47].

In a small series of 5 patients with advanced PAD and foot ulcers, intramuscular (gastrocnemius muscle) BMC transplantation achieved substantial improvement in pain intensity and pain-free walking distance in all of them after 12 months [48]. Complete ulcer healing was achieved in 3 and partial healing in one patient [48]. There was a progressive improvement of ABI and TcPO<sub>2</sub> over 12 months: at the end of this period, the average ABI rose from 0.41 to 0.83 ( $p < 0.05$ ) and the average TcPO<sub>2</sub> from 18.8 to 37.5 mmHg [48]. Duplex ultrasonography showed improvement in one patient, while angiography detected new collaterals in 3 patients [48].

Eighteen patients with advanced PAD (staged Fontaine III/IV) were recruited in another long-term study with 18-month follow-up. The control group included 18 matched patients taking maximal drug therapy [49]. In the treatment arm, patients received BMCs in two intra-arterial doses and, concurrently, daily antioxidants and L-arginine. Among BMC-treated patients, mean walking distance started to increase at 3 months and exhibited further improvement at 18 months ( $p < 0.05$ ) [49]. Significant ( $p < 0.05$ ) improvement in ABI was seen in 10/18 patients at 3 months and in 12/18 patients at 18 months [49]. Ischaemic ulcers improved in 13/18 patients after 6-12 months. Amputation rates were 13.3% (2/18) in BMC-treated patients and 55.6% (10/18) in controls ( $p = 0.014$ ) [49].

A total of 37 patients suffering from PAD staged Fontaine IV with an ulcerated limb (including diabetic foot) receiving BMCs intramuscularly were recruited in a further trial [50]. Efficacy parameters included toe pressure, toe-pressure index (TBI), ABI, TcPO<sub>2</sub>, LDI, skin perfusion pressure, wound healing and amputation rates. Limb salvage was achieved in 30 patients (81%) and amputation rate was 19% (7 patients) [50]. In the limb salvage group, significant ( $p < 0.05$ ) improvements in toe pressure, TBI, LDI and TcPO<sub>2</sub> were noted [50]. Significant ( $p < 0.05$ ) improvement in pain-free walking distance at treadmill testing was also shown in a study of 42 subjects with chronic PAD involving the femoropopliteal-tibial segment by intramuscular BMC administration [51].

To evaluate the long-term efficacy of BMC transplantation in subjects with PAD without option for revascularisation or with unsuccessful revascularisation, the BONE Marrow Outcome Trial in Critical Limb Ischemia (BONMOT-CLI) was designed [52]. This is a double-blinded, 1:1 randomised, placebo-controlled multi-centre study enrolling patients from 4 German centres. Patients will be randomised to autologous BMCs (expected 45 patients) injected at 40 sites into the ischaemic limb or sham bone marrow aspiration and 40 saline injections [52]. The composite primary endpoint of major amputation or persisting critical limb ischaemia will be evaluated at 3 months. Subjects will be then followed up for up to two years. Secondary endpoints will include death, changes in perfusion, quality of life, pain-free walking distance, minor amputations, wound healing, collateral density and cancer incidence [52].

#### 4.1.2 BMMNCs

In 2002, the Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators recruited 25 patients with unilateral leg ischaemia (group A), who received BMMNCs into the gastrocnemius muscle and saline into the less ischaemic limb, as well as 22 patients with bilateral leg ischaemia (group B), who randomly received BMMNCs in one leg and peripheral blood-mononuclear cells in the other as a control [53]. Efficacy parameters included ABI, TcPO<sub>2</sub> and rest pain. In group B, the BMMNC-treated limb exhibited significant increases in ABI, as compared to the contralateral limb, by 0.09 (CI 0.06-0.11,  $p < 0.0001$ ), in TcPO<sub>2</sub> by 13 mmHg (CI 9-17,  $p < 0.0001$ ) and in pain-free walking time by

1.2 min (CI 0.7-1.7,  $p=0.0001$ ) [53]. Pain intensity was also reduced by -0.85 (-1.6 to -0.12,  $p=0.025$ ) in comparison to the contralateral limb [53]. Similar improvements were seen in the BMMNC-treated vs. saline-treated extremity in group A. The new therapy was well tolerated. Two patients in group A died after myocardial infarction, but this was unrelated to treatment [53].

Kirana et al. [54] described a 60-year old type 2 diabetic patient with infected gangrenous ulcers of the 3<sup>rd</sup> and 4<sup>th</sup> left toes. The patient has severe stenosis of anterior tibial, posterior tibial and dorsalis pedis arteries, leading to critical ischaemia. After amputation of the affected toes, he received intramuscular BMMNC transplantation. After 20 weeks, the stump had completely healed, and there were improvements in ABI, Laser Doppler reactive hyperaemia and TcPO<sub>2</sub> [54].

Higashi et al. [55] studied the effect of BMMNCs in 7 patients with PAD. Treatment improved ABI (from  $0.33\pm0.21$  to  $0.39\pm0.17$ ,  $p=0.06$ ), TcPO<sub>2</sub> (from  $28.4\pm11.5$  to  $36.6\pm5.2$  mm Hg,  $p=0.03$ ), and pain-free walking time (from  $0.8\pm0.6$  to  $2.9\pm2.2$  minutes,  $p=0.02$ ) [55]. Moreover, it improved endothelial function, evaluated by blood flow in response to acetylcholine (from  $19.3\pm6.8$  to  $29.6\pm7.1$  mL/min per 100 mL,  $p=0.002$ ) [55]. In the same year, two other small studies the efficacy of BMMNCs. The first work showed that these cells improved blood flow in 12 patients with PAD staged Fontaine IV [56]. Specifically, there was an increase in pain-free walking time (from  $140\pm53$  s to  $451\pm74$  s,  $p=0.034$ ), ABI (from  $0.65\pm0.08$  to  $0.73\pm0.07$ ,  $p=0.055$ ) and perfusion index (proximal area from  $1.32\pm0.10$  to  $1.56\pm0.11$ ,  $p=0.007$ ) [56]. Perfusion scintigraphy could identify new collaterals after BMMNC transplantation [56]. The second work showed increased ABI (from  $0.54\pm0.47$  to  $0.61\pm0.50$ ,  $p<0.05$ ) and TcPO<sub>2</sub> ( $28.4\pm15.4$  mmHg to  $37.1\pm24.4$  mmHg) following BMMNC transplantation in 8 patients with PAD [57].

A group from the Heinrich Heine University of Düsseldorf, Germany has performed a series of small studies enquiring into the efficacy of BMMNCs. A 62-year old man with PAD staged Fontaine IIb received combined intra-arterial and intramuscular transplantation of BMMNCs [58]. After 10 weeks, there was a 7-fold improvement of walking distance, a greater than 50% increase of tissue oxygen saturation and a 24% increment of ABI on exercise [58]. Then, 10 patients received intra-arterial (into the common femoral artery) and intramuscular (into the muscles of the thigh and the lower leg) BMMNC transplantation [59]. After 2 months, pain-free walking distance increased significantly ( $p<0.05$ ) in all patients. Moreover, significant ( $p<0.05$ ) improvement of ABI, capillary-venous oxygen saturation and parameters of venous occlusion plethysmography were observed [59]. A 63-year-old type 2 diabetic patient with severe intermittent claudication and a recalcitrant ulcer in the right hallux was treated with intra-arterial and intramuscular BMMNC transplantation [60]. At 8 weeks, the ulcer had healed completely. Six months later, there were significant ( $p<0.05$ ) increments in the claudication-free walking distance by >100% and in resting blood flow on venous occlusion plethysmography by 23% [60]. The next study in 8 patients with PAD staged Fontaine II or III demonstrated a significant ( $p<0.05$ ) 3.7-fold increment in pain-free walking distance [61]. ABI on exercise (from  $0.62\pm0.17$  to  $0.77\pm0.15$ ,  $p=0.018$ ), capillary-venous oxygen saturation (from  $50\pm15$  to  $62\pm6\%$ ,  $p=0.027$ ) and venous occlusion plethysmography (from  $4.6\pm1.7$  to  $6.5\pm1.7$  ml/100 ml tissue/min,  $p<0.05$ ) exhibited significant improvements as well [61]. Finally, 13 subjects with chronic PAD staged Fontaine IIb received combined intra-arterial and intramuscular BMMNC transplantation [62]. After 2 months, the pain-free walking distance increased significantly by more than 300% (from  $147\pm90$  to  $500\pm614$  m,  $p=0.001$ ). ABI rose, both at rest (from



0.66±0.18 to 0.80±0.15,  $p=0.003$ ) and on exercise (from 0.64±0.19 to 0.76±0.16,  $p=0.006$ ) [62]. The authors also found significant improvements in capillary-venous oxygen saturation (from 56±14 to 63±5,  $p=0.021$ ) and venous occlusion plethysmography (from 2.1±0.7 to 2.5±0.7,  $p=0.009$ ). Importantly, beneficial effects were sustained at 13 months [62].

A4 mixed series of 35 patients (30 with ischaemic diabetic foot, 2 with PAD and 3 with TAO) received BMMNC transplantation after bone marrow mobilisation with GcSF [63]. Pain was entirely relieved in 94.7% and improved in 97.1% of patients [63]. Numbness was alleviated in 93.3% of patients. Claudication-free walking distance was prolonged in all subjects, while 47.9% exhibited a significant ( $p<0.05$ ) increase in ABI and 92.3% a significant ( $p<0.05$ ) increase in TcPO<sub>2</sub> [63]. Ulcer healing was accomplished in 9.1% and ulcer area reduction in 27.3% [63]. Amputation rate was 6.3%. New collateral vessels were identified by angiography in 91.2%. Complications included transient fever and mild fatigue in one patient and acute myocardial infarction in one patient. The latter occurred 7 days following transplantation and easily recovered with treatment [63].

These authors then compared intramuscular (group A,  $n=16$ ) with intra-arterial (group B,  $n=16$ ) BMMNC injection [64]. Efficacy parameters included rest pain, coldness, ABI, intermittent claudication, TcPO<sub>2</sub> and angiography. Treatment was equally efficacious in both groups [64]. Rest pain was improved in 76.5% of group A and in 93.3% of group B patients. Coldness improved in 100% of patients from both groups. ABI increased in 44.4% of group A and in 41.2% of group B patients [64]. Limb salvage was achieved in 83.3% of group A and in 94.1% of group B patients [64]. TcPO<sub>2</sub> increased to  $\geq 20$  mmHg in 20 limbs, while a rich new collateral network was identified in 9/15 limbs which underwent angiography. There were 2 deaths from heart failure [64].

Longer data from this group have recently become available [65]. A total of 65 patients with PAD have received BMMNCs: 12 patients were transplanted 2-4 times and 53 patients only once [65]. Mean follow-up was 21.5 months (range 8-56) [65]. In both treatment groups, coldness improved in all patients, while there were significant ( $p<0.05$ ) increments in ABI and TcPO<sub>2</sub>. Overall efficacy was 70.8% and the recurrence rate was 10.7%. Response duration was over 12 months in 91.3% of patients, over 24 months in 52.2% and over 37 months in 26.1%. Efficacy was significantly ( $p<0.001$ ) higher in subjects transplanted 2-4 times (100%) than in those transplanted once (64.2%) [65]. Mortality rate was 12.3%: 5 patients died of myocardial infarction and heart failure, and 3 died of cerebral infarction [65].

Twelve patients received BMMNC transplantation by two different techniques (either sorted on a blood cell separator or isolated by density gradient on Ficoll-Hypaque) [66]. Both modalities were equally effective in improving ABI at rest, oxygen saturation, pain-free walking time and rest pain intensity. Improvement was sustained at 24 weeks and limb salvage rate was 41.67% [66]. In another small series, 7 patients (3 with TAO and 4 with PAD undergoing haemodialysis) received mononuclear cell transplantation (BMMNC in 6, PBMNC in 1) into the gastrocnemius and quadriceps femoris muscles [67]. Patients with TAO exhibited improvements in painful symptoms, ABI, TcPO<sub>2</sub> and thermography, while patients with PAD did not respond [67]. It was concluded that transplantation was more effective in TAO than in PAD, but this must be interpreted with caution, given the very small number of patients [67].

Van Tongeren et al. [68] compared combined intra-arterial plus intramuscular ( $n=12$ ) to exclusive intramuscular ( $n=15$ ) BMMNC transplantation. Efficacy was assessed at 1, 6 and 12 months by means of limb salvage, pain-free walking distance, ABI and pain scores [68].

Both modalities were equally effective. Pain-free walking distance improved from  $81 \pm 56$  m to  $257 \pm 126$  m at 6 months ( $p=0.0002$ ). Mean ABI increased by 23% after 6 months ( $p=0.01$ ) and pain score was reduced up to 50% ( $p=0.001$ ). Two patients in the combined treatment group vs. 7 patients in the intramuscular group ( $p=0.17$ ) required amputation [68].

In the same year, the 3-year outcomes of the TACT (Therapeutic Angiogenesis using Cell Transplantation) follow-up study were published [69]. Primary endpoints comprised mortality and amputation-free interval. Median follow-up was 25.3 months (range 0.8-69.0 months) and 3-year survival rates were 80% (CI 68-91) in PAD ( $n=74$ ) and 100% in TAO ( $n=41$ ). Amputation-free rates were 60% (CI 46-74) and 91% (CI 82-100), respectively [69]. At 2 years, ABI and  $TcPO_2$  had not changed significantly, but there was a sustainable significant improvement in pain scale, ulcer size and pain-free walking distance [69].

Ten patients with end-stage PAD underwent 2 BMMNC transplantations, while 10 matched patients served as controls [70]. In the treatment arm, there was a significant ( $p<0.05$ ) improvement in ABI, claudication-free walking distance and capillary density, which were maintained at 12 months [70]. Eight patients with critical limb ischaemia (CLI) and no alternative treatment option received BMMNC transplantation into the gastrocnemius muscle [71]. Pain, angiography and non-invasive vascular workup were evaluated. At 4 months, pain was reduced in 5 patients. At 8 months, 5 patients could be evaluated and showed stability or insignificant improvement [71].

Twenty-four patients with CLI received intra-arterial BMMNC transplantation [72]. After 12 months, all patients survived and only 2 of them had undergone amputation. Ulcer healing rate was 78% and median Fontaine stage had improved from 3.5 to 2 ( $p<0.0001$ ) [72]. Collateral vessel formation had improved by 1.13 and 1.3 points on a four-point semiquantitative scale in calf and foot, respectively ( $p<0.0001$ ) [72]. Impressively, significant improvements were reported in all items of the SF-36 quality of life questionnaire [72].

De Vriese et al. [73] included 16 very old patients (mean age  $78 \pm 2$  years) with CLI and substantial comorbidities (hypertension, smoking, diabetes, hypercholesterolaemia and uraemia), who underwent intramuscular BMMNC transplantation.  $TcPO_2$  improved from  $0.51 \pm 0.11$  to  $0.86 \pm 0.03$  mm Hg ( $p<0.001$ ) after 12 weeks, whereas ABI showed no significant change ( $0.42 \pm 0.15$  vs.  $0.59 \pm 0.1$ ,  $p=0.23$ ) [73]. On digital subtraction angiography, the number of collateral vessels increased by  $0.89 \pm 0.86$  ( $p=0.33$ ), but capillary surface area on gastrocnemius muscle biopsy increased from  $0.61 \pm 0.07\%$  to  $2.38 \pm 0.73\%$  ( $p<0.05$ ) [73]. Two patients died of gangrene, 3 patients were amputated and one patient required by-pass surgery. Two further patients died of unrelated causes [73]. Of note, symptomatic relief was mainly achieved in patients with less severe ischaemia [73]. Thus, despite some objective improvement in vascular parameters, BMMNC administration was only associated with very modest overall improvement in these high-risk patients.

More recently, 51 patients with limb-threatening CLI facing risk of major amputation received BMMNCs intramuscularly [74]. This treatment was offered after unsuccessful or impossible revascularisation procedure and optimal medical therapy. Limb salvage was 59% at 6 months and 53% at the end of the study (mean follow-up  $411 \pm 261$  days) [74]. At 6 months, ABI increased from  $0.33 \pm 0.18$  to  $0.46 \pm 0.15$  ( $p=0.005$ ) and  $TcPO_2$  increased from  $12 \pm 12$  to  $25 \pm 15$  mmHg ( $p=0.001$ ) in patients with limb salvage, but not in those ultimately amputated [74]. The former were also downstaged from a mean Rutherford category of 4.9 to 3.3 ( $p=0.0001$ ). Wound area was reduced from  $11.6 \pm 20$  to  $4.4 \pm 11$  cm<sup>2</sup>. Median walking distance improved from 0 to 40 m, but only in those escaping amputation. Finally, analgesic requirement dropped by 62% [74].

A further work looked at the short-term results of dual intramuscular and intra-arterial autologous BMMNC transplantation in 9 patients facing the risk of lower-extremity amputation [75]. Eight patients had rest pain, 7 had diabetes mellitus and 8 recalcitrant ulcers. Efficacy parameters comprised ABI, rest pain, ulcer healing and amputation. The primary composite endpoint was defined as improved ABI, relief of rest pain, ulcer healing and absence of major amputations [75]. Overall success rate was 33.3% (3 patients), while success in at least one of the 4 components of the primary endpoint was noted in 5 additional patients (55.6%) [75]. ABI exhibited a non-significant improvement by 0.12 (dorsalis pedis artery) and 0.08 (posterior tibial artery). Three patients (33.3%) sustained major amputations. Those remaining free from amputations showed improvement in patient severity and could be downstaged by at least one level in Rutherford and Fontaine classifications at a mean follow-up of 7.8 months [75]. Complete ulcer healing was accomplished at 3 months in all ulcerated patients not needing amputation. These short-term findings favoured BMMNC transplantation for limb salvage in patients with severe PAD, but it must be borne in mind that patient numbers were small [75].

| Authors                     | Number of Patients | Type of Cells     | Clinical outcomes  |
|-----------------------------|--------------------|-------------------|--|
| Nizankowski et al. [46]     | 10                 | BMCs              | Improvement of symptoms, increased blood flow (LDI, TcPO <sub>2</sub> )  |
| Gu et al. [47]              | 22                 | BMCs              | Improvement of symptoms (pain, cold sensation), increase in ABI and TcPO <sub>2</sub> , new collateral vessels |
| Napoli et al. [49]          | 18                 | BMCs              | Increase in ABI and walking distance, ulcer healing, reduction of amputation rates                             |
| Procházka et al. [50]       | 37                 | BMCs              | Improvement in toe pressure, TBI, LDI and TcPO <sub>2</sub>  |
| Korymasov et al. [51]       | 42                 | BMCs              | Improvement in pain-free walking distance  |
| Tateishi-Yuyama et al. [53] | 25                 | BMMNCs vs. PBMNCs | Increase in ABI, TcPO <sub>2</sub> and walking distance  |
| Higashi et al. [55]         | 7                  | BMMNCs            | Increase in ABI, TcPO <sub>2</sub> , endothelial function and walking distance                                 |
| Miyamoto et al. [56]        | 12                 | BMMNCs            | Increase in ABI, perfusion index and walking distance  |

| Authors                  | Number of Patients                            | Type of Cells | Clinical outcomes   |
|--------------------------|---|---------------|---|
| Bartsch et al. [62]      | 13  | BMMNCs        | Long-term improvement in walking distance, ABI and oxygen saturation  |
| Gu et al. [63]           | 35 (30 ischaemic diabetic foot, 2 PAD, 3 TAO) | BMMNCs        | Improvement in pain, numbness and walking distance, increase in ABI and TcPO <sub>2</sub> , ulcer healing, new collateral vessels                 |
| Gu et al. [64]           | 32  | BMMNCs        | Improvement in symptoms, ABI and TcPO <sub>2</sub> , limb salvage, new collateral vessels   |
| Gu et al. [65]           | 65  | BMMNCs        | Long-term improvement in coldness, ABI and TcPO <sub>2</sub> , new collateral vessels   |
| Van Tongeren et al. [68] | 27  | BMMNCs        | Improvement in walking distance and ABI, reduced amputation rates   |
| Matoba et al. [69]       | 115 (74 PAD, 41 TAO)                          | BMMNCs        | Long-term improvement in pain scale, ulcer size and walking distance, reduced amputation rates  |
| Chochola et al. [72]     | 24  | BMMNCs        | Ulcer healing, new collateral vessels, Fontaine downstaging, improved quality of life   |
| De Vriese et al. [73]    | 16  | BMMNCs        | Increased capillary surface area on muscle biopsy, increase in TcPO <sub>2</sub> , insignificant increase in ABI and number of collateral vessels |
| Amann et al. [74]        | 51  | BMMNCs        | Limb salvage, increase in ABI and TcPO <sub>2</sub> ,   |

Table 1. Major clinical studies with bone marrow-derived stem cell therapy



Finally, in a phase I clinical trial, 10 patients with advanced PAD (Fontaine stages IIb to IV) received a cell product consisting of autologous BMMNCs and MSCs [76]. ABI improved significantly (from  $0.34 \pm 0.19$  to  $0.69 \pm 0.18$ ,  $p < 0.002$ ) at 2 months. Claudication-free walking time increased significantly ( $p < 0.05$ ) by  $3.48 \pm 1.72$  folds at 6 months [76]. Significant ( $p < 0.05$ ) improvements were also seen in  $^{99m}\text{Tc}$ -TF perfusion scintigraphy scores and in quality of life scores at 6 months [76].  $\text{TcPO}_2$  rose insignificantly ( $p = 0.067$ ) from  $33 \pm 6$  mm Hg to  $46 \pm 10$  mm Hg at 6 months, but angiographic score increased from  $0.90 \pm 0.30$  to  $1.89 \pm 0.78$  ( $p = 0.002$ ) [76].

## 4.2 Stem cells from peripheral blood

### 4.2.1 PBMNCs

The main cell population used is PBMNCs, with or without GcSF mobilisation. Inaba et al. [77] included 7 patients with PAD, who received GcSF subcutaneously for 5 days and then intramuscular PBMNC transplantation. Pain was relieved as early as at 3 days [77]. Maximum pain-free walking distance was increased by day 7. The heel ulcer completely healed in one patient. These improvements were sustained at 12 months. Improvements in ABI and angiographic findings were minor [77].

Ishida et al. [78] examined the feasibility and safety of PBMNC administration after GcSF mobilisation in 6 patients (1 with PAD, 5 with TAO). A slight increase in ABI and ulcer area were seen in 4 and 3 patients, respectively [78]. Mean walking distance significantly increased from 203 m to 559 m ( $p = 0.031$ ) at 4 weeks and was sustainable for 24 weeks. There was also a significant improvement in physiological functioning, as evaluated by the SF-36 questionnaire on quality of life [78]. No serious adverse events were noted.

In 92 patients with CLI, intramuscular PBMNC administration after GcSF mobilisation was most effective for non-diabetic non-dialysis subjects in terms of averting amputations [79]. In those with diabetes and/or undergoing haemodialysis, treatment was efficacious for milder ischaemia (up to Fontaine stage III), but not for advanced disease (staged Fontaine IV) [79]. Indeed, most amputations ( $n = 37$ ) were carried out in Fontaine staged IV diabetic or dialysis patients. Characteristically, amputation rate was as high as 71% in Fontaine staged IV diabetic patients on haemodialysis [79]. Interestingly, serum VEGF levels increased in all patients (mean increase 176%), regardless of clinical outcome.

In 15 patients with CLI, intramuscular PBMNC administration after GcSF mobilisation induced significant ( $p < 0.05$ ) increases in mean ABI (from 0.3 to 0.46), mean pain-free walking distance (from 0.15 to 0.72 km) and mean maximal walking distance (from 0.96 to 2.13 km) at 12 months [80]. Moreover, 5/6 foot ulcers healed completely.

Two studies compared PBMNC to BMMNC transplantation. Gu et al. [19] carried out this comparison in 42 patients with unilateral lower extremity ischaemia (28 with ischaemic diabetic foot, 8 with TAO and 6 patients with PAD). Each therapy was administered to 21 patients. No difference was seen between the two groups [19]. All but one ulcers healed. At 4 weeks, pain was relieved in 88.2% of patients in the BMMNC and in 89.5% of patients in the PBMNC group [19]. Cold sensation was relieved in 94.4% of BMMNC and 94.7% of PBMNC patients. Numbness was improved in 69.2% of BMMNC and 66.7% of PBMNC patients. ABI increased in 38.1% of BMMNC and 33.3% of PBMNC patients.  $\text{TcPO}_2$  increased in 85.7% of BMMNC and 90.5% of PBMNC patients. New collateral vessels were identified in 83.3% of BMMNC and 77.8% of PBMNC patients. Wound healing was noted in 60.0% of BMMNC and 66.7% of PBMNC patients, and amputation rate was 9.1% in each group. Follow-up was extended to a mean of 8 months (range 3-15) in 40 patients [19]. At the end of

follow-up, painful symptoms improved in 75.0% of BMMNC and 70.0% of PBMNC patients, ABI increased in 60.0% of BMMNC and 65.0% of PBMNC patients. TcPO<sub>2</sub> increased in 80.0% of BMMNC and 75.0% of PBMNC patients. Finally, new collateral vessels were detected in 90.0% of BMMNC and 84.6% of PBMNC patients [19].

Similarly, 150 patients with PAD were randomised to PBMNC (n=76) or BMMNC (n=74) and followed for 12 weeks [20]. In the PBMNC group, improvements in ABI ( $p<0.0001$ ), skin temperature ( $p=0.028$ ), and rest pain ( $p<0.0001$ ) were significantly more pronounced in comparison to the BMMNC group [20]. However, no between-group difference was found in terms of pain-free walking distance, TcPO<sub>2</sub>, ulcers, and amputation rates. Thus, while both treatment options were effective, PBMNC administration yielded higher overall efficacy [20].

Finally, Zhang et al. [23] studied 15 patients (10 with PAD, 5 with TAO) suffering from severe ischaemia affecting the popliteal and distal arterial segment who could not be surgically revascularised. Four patients were Fontaine stage II, five patients were stage III, and six patients were stage IV. Three patients had diabetes mellitus and one had chronic renal failure [23]. All patients received PBMNCs intramuscularly. After two and 12 months, ABI, TcPO<sub>2</sub>, claudication-free walking distance and pain intensity improved significantly ( $p<0.005$ ) [23]. A significant increase in new vessel formation was also evidenced by angiography at 24 weeks. No adverse events were noted.

#### 4.2.2 Other stem cells

Seven patients with CLI (Rutherford stages 4 or 5) were treated with an intra-arterial infusion of autologous CPCs isolated from peripheral blood following GcSF mobilisation [81]. After 12 weeks, a 30-fold increase in pain-free walking distance from  $6.4\pm 12.5$  to  $195\pm 196$  m ( $p=0.016$ ) was evident. At the same time, pain intensity was significantly reduced (from  $8\pm 1$  to  $2\pm 2$ ;  $p=0.001$ ). There were also significant increments in ABI (from  $0.48\pm 0.09$  to  $0.64\pm 0.11$   $p=0.001$ ) and TcPO<sub>2</sub> (from  $15\pm 10$  to  $35\pm 9$  mmHg,  $p=0.001$ ) [81]. Moreover, a 5-fold increase in flow-dependent vasodilation (from  $0.9\pm 0.3$  to  $5.0\pm 1.4\%$   $p=0.016$ ) and a 140% increase in adenosine-dependent flow reserve (from  $3.6\pm 1.3$  to  $4.9\pm 1.7$ ,  $p=0.004$ ) in the superficial femoral artery were documented. Local or systemic adverse events were not seen [81].

In 6 patients with CLI due to PAD involving the infrapopliteal segment, intramuscular injections of non-mobilised peripheral blood angiogenic cell precursors (NMPB-ACPs) were carried out [82]. Five patients showed significant improvement in blood flow (ABI, TcPO<sub>2</sub>) and 4 had complete healing of ulcers or amputation stumps. However, major amputation could finally not be avoided in 2 patients [82]. Obviously, more experience with this treatment is needed.

### 5. Safety issues

The vast majority of studies suggests that stem cell therapy for PAD is very safe. Local, injection-related side effects are, indeed, extremely rare. As regards systemic toxicity, there have been concerns with the use of GcSF, given that this growth factor has been reported to occasionally cause coronary ischaemia [83] or acute arterial thrombosis [84]. However, safety profiles of studies using GcSF have not been worse than those without GcSF.

In some studies, deaths have been reported. These were mostly due to acute myocardial infarction [53, 65, 69] congestive heart failure [64, 65, 69] and stroke [65, 69], while perforation peritonitis [69], sepsis [69] and suicide [69] have been reported as exceptionally

| Authors              | Number of Patients                            | Type of Cells                | Clinical outcomes  |
|----------------------|---|------------------------------|--|
| Inaba et al. [77]    | 7   | PBMNCs, with or without GcSF | Increased walking distance, minor improvements in ABI and angiographic findings  |
| Ishida et al. [78]   | 6 (1 PAD, 5 TAO)                              | PBMNCs with GcSF             | Increase in walking distance, slight improvement in ABI and ulcer area   |
| Kawamura et al. [79] | 92  | PBMNCs with GcSF             | Reduced amputations, mostly in non-diabetic non-dialysis patients  |
| Zhang et al. [80]    | 15  | PBMNCs                       | Increased walking distance and ABI, ulcer healing  |
| Gu et al. [19]       | 42 (28 ischaemic diabetic foot, 8 TAO, 6 PAD) | PBMNCs vs. BMMNCs            | Equal efficacy: ulcer healing, improvement of symptoms, increase in ABI and TcPO <sub>2</sub> , new collateral vessels   |
| Huang et al. [20]    | 150   | PBMNCs vs. BMMNCs            | PBMNC administration: higher overall efficacy. Improvement in ABI, skin temperature, rest pain, walking distance, TcPO <sub>2</sub> , ulcers, and amputation rates (both treatments) |
| Zhang et al. [23]    | 15 (10 PAD, 5 TAO)                            | PBMNCs                       | Long-term improvement in ABI, TcPO <sub>2</sub> , walking distance and pain, new collateral vessels  |
| Lenk et al. [81]     | 7   | CPCs with GcSF               | Reduced pain, increase in walking distance, ABI, TcPO <sub>2</sub> , flow-dependent vasodilation and adenosine-dependent flow reserve  |

Table 2. Major clinical studies with peripheral blood-derived stem cell therapy

rare causes. However, death events were never clearly related to stem cell therapy. Conversely, some of them could be characterised as clearly unrelated [53, 65]. Indeed, patients recruited in stem cell studies had severe or even limb-threatening vascular disease with considerable comorbidity. Such patients are generally a priori anticipated to suffer further vascular events, mainly in the coronary or cerebral arteries [1, 2, 4, 5]. Of note, in the study with the longest follow-up and most information on safety [69], mortality rate was 14.9% in PAD and 0% in TAO. Thus, caution is needed before attributing this mortality to stem cell treatment, and more information is required.

Serious adverse events have included acute myocardial infarction, stroke, post-surgery restenosis of coronary arteries, sepsis and peritonitis, while mild adverse events have included transient fever and myalgia [69]. Matoba et al. [69] have provided the most detailed and long-term data. Again, these adverse events were only observed in PAD and not in TAO, casting doubt on their relation to treatment. Regrettably, there was no control group to compare event rates.

In summary, stem cell transplantation appears to be generally safe. Deaths and adverse events have never been unambiguously linked to treatment. Nonetheless, it should not escape our notice that patient series have been very small, some studies only presenting case series of up to 10 subjects [45, 46, 48, 54, 58-62, 75-78, 82]. Follow-up has also been relatively short, in most studies less than 1 year. More importantly, event rates could not be compared to a control group. Hence, additional data is eagerly awaited to confirm or refute this safety profile.

## 6. Clinical implications

Available evidence suggests that stem cell therapy should be considered for those patients with severe PAD who are poor candidates for revascularisation. The question then to ask would be: bone marrow-derived or peripheral blood-derived stem cells? Not to be underestimated, the latter is easier to perform and, theoretically, offers the incremental advantage that it might be repeated. Moreover, it does not appear to be inferior in efficacy, but more comparative data is required. At least as regards mononuclear cells, PBMNCs show comparable [19] or even superior [20] efficacy in comparison to BMMNCs. If comparable efficacy is generalised for all bone marrow-derived vs. peripheral blood-derived stem cells, then the individual decision may rest with the treating physician, depending on previous experience and availability issues.

BMCs, BMMNCs and PBMNCs are the main cell types used [19, 20, 23, 29, 47, 50, 55, 56, 63-65, 68, 69, 73, 74, 77, 78, 80, 81], and there is no clear superiority of one cell type over the others. Administration has been intramuscular into the ischaemic extremity in the vast majority of trials. Combined intra-arterial and intramuscular administration has also been used [58-60, 62]. Combined intra-arterial and intramuscular BMMNC transplantation has been shown to be equally effective to intramuscular transplantation [68]. Gu et al. [64] showed equal treatment efficacy for intra-arterial vs. intramuscular BMMNC transplantation. Arguably, intramuscular administration may, for the time being, constitute the route of choice, since it has been more extensively studied.

The beneficial effect of stem cell therapy has been evaluated by diverse outcomes. These include alleviation of pain [19, 23, 46, 47, 51, 63, 69], increased walking distance [19, 20, 23, 47, 51, 53, 55, 56, 63, 68, 69, 77, 78, 80], improved ABI and TcPO<sub>2</sub> [19, 20, 23, 47, 53, 55, 56, 50, 63, 68, 69, 73, 74, 77, 78, 80, 81], but also hard endpoints, notably ulcer healing [19, 20, 47, 49,



63, 69, 74, 78, 80], quality of life [72] and limb salvage [20, 49, 68, 69, 79]. Moreover, new collateral vessels have been identified on angiography [19, 23, 47, 63, 65, 72, 73]. Thus, despite the variety of efficacy parameters in the individual works, it is true that studies converge to suggest an objectively demonstrable and clinically meaningful benefit.

Importantly, however, long-term efficacy needs to be better studied as well. Indeed, follow-up in most studies has been up to one year and few works have provided long-term data [23, 62, 65, 69]. Based on the latter, treatment-induced improvements in painful symptoms, walking distance and blood flow parameters (ABI, TcPO<sub>2</sub>) appear to be, at least in part, sustainable at 2-3 years [23, 62, 65, 69]. If long-term efficacy becomes finally established, the argument in favour of stem cell therapy for severe inoperable PAD will be strongly enhanced.

Two further issues deserve careful consideration. First, safety profile appears, as already discussed, very good, but more information is needed. Ideally, we need a robust analysis balancing improvement in clinical outcomes to adverse event rates. In this context, it is extremely important to define the number needed to treat (NNT) and the number needed to harm (NNH) before attempting more widespread use of this treatment option. NNT and NNH could even vary, according to the type of patient population (e.g. PAD, CLI, TAO, acute CLI, elderly patients with substantial comorbidity and so on) [69]. However, this information is vital for clinical decisions outside randomised clinical trials. Secondly, stem cell therapy is an expensive treatment and its cost-effectiveness has not been determined. Thus, a detailed cost-benefit analysis is desirable.

Last, but not least, stem cell therapy has hitherto only been explored as a salvage therapy in severe lower extremity ischaemia not responding to standardised treatment. Such approach has precluded comparison with revascularisation. Still, given the promising results in clinical benefits achieved with stem cell transplantation, it may be worthwhile to consider this therapeutic option earlier in the course of PAD, instead of reserving it for patients with no other choice. Earlier utilisation of stem cell therapy might thus yield even better results. Moreover, it might be combined with revascularisation, if both are used in the appropriate time frame. These are important aspects for future investigation.

## 7. Conclusions

Stem cell therapy is emerging as a promising treatment option for patients with PAD who are poor candidates for established treatment, including revascularisation and best medical therapy. There are two options, bone marrow-derived and peripheral blood-derived stem cells. Theoretically, transplantation of peripheral blood-derived stem cells offers the additional advantage that it can be repeated, but this potential has not been utilised. In the vast majority of cases, stem cell administration has been intramuscular into the ischaemic limb, but intra-arterial administration appears to be equally efficacious. The number of cells that need to be transplanted merits more precise study. Finally, while the new treatment appears generally safe, more data from larger patient series and longer follow-up are awaited for confirmation.

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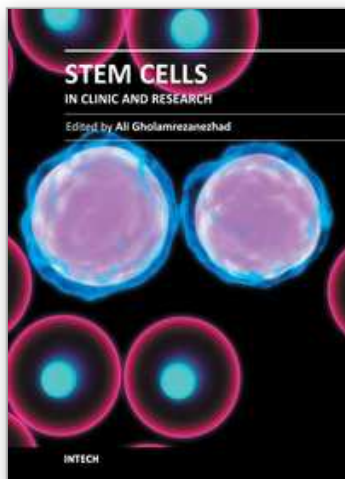


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## **Stem Cells in Clinic and Research**

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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigational more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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