Chapter 11
Mesenchymal Stem Cell and Endothelial Progenitor Cell Transplantation for Buerger's Disease

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Thromboangiitis obliterans (TAO), also known as Buerger's disease, was first described by von Winiwarter in a person in 1879 [1]. In 1908, Leo Buerger published a detailed description of the pathological findings on 11 amputated limbs and named the disease [2]. It is a nonatherosclerotic inflammatory disorder of unknown etiology that affects small- and medium-sized vessels of the extremities. TAO may also affect gastrointestinal, cerebrovascular, coronary, and renal arteries in some cases [3, 4].

Although smoking is the strongest risk factor in the development and progression of TAO, the specific mechanism of tobacco in the etiopathogenesis of TAO is not fully understood. It is not known which exact components of tobacco are involved in the pathogenesis of TAO. Hereditary factors (related to specific human leukocyte antigen haplotypes) may also contribute to the development of TAO. Endothelial dysfunction is associated with inflammation and thereby impaired endothelium-dependent vasorelaxation [4]. Most importantly a body of literature has addressed TAO as an autoimmune disease [5]. However, the exact etiology of TAO is still elusive.

A staging system for clinical symptoms was proposed by Rutherford (Table 11.1).

There is no standard treatment for TAO. The most effective intervention for TAO is smoking cessation as smoking is the strongest risk factor for TAO. Platelet inhibitors, such as aspirin and clopidogrel, may reduce secondary events in TAO patients with atherosclerotic disease. Vasodilators, such as amlodipine, nifedipine, and verapamil, can alleviate symptoms by dilating vessels proximal to the stenotic or occlusive lesion. Other drugs that have been proven beneficial include pentoxifylline,

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Table 11.1 Rutherford classification

Grade	Category	Clinical
0	0	Asymptomatic
I	1	Mild claudication
I	2	Moderate claudication
I	3	Severe claudication
II	4	Rest pain
III	5	Ischemic ulcer not exceeding digits
IV	6	Severe ischemic ulcer or gangrene

Fig. 11.1 Photographs showing healed amputation stumps

cilostazol naftidrofuryl, levocarnitine, arginine, buflomedil, ketanserin, niacin, lovastatin, bosentan, treprostinil, and prostacyclin derivatives. Overall, the long-term outcomes of the pharmacological inventions are unfavorable. Administration of growth factors and gene vectors for vascular endothelial growth factor (VEGF) gene to improve endothelial cell proliferation, migration, and blood vessel formation in the ischemic limb has also been reported with encouraging results. Spinal cord stimulators may modulate painful stimuli and alleviate pain in severe TAO. Surgical revascularization is rarely possible for TAO patients due to the diffused vascular damage of the distal vessels. Endovascular therapy may be technically challenging because of the prevalent location of lesions in distal vessels. Thus other innovative therapies are crucial to decrease amputation rate or even avoid amputation (Fig. 11.1).

A promising treatment for critical limb ischemia is stem cell therapy, in which stem cells are injected into the affected area to solicit the so-called therapeutic angiogenesis. Stem cells are undifferentiated cells that theoretically have the ability to differentiate into a specialized adult cell type in a specific tissue in the human body. Multipotent adult stem cells are found in differentiated tissues. The first reported trial of therapeutic angiogenesis using stem cells was the therapeutic angiogenesis by cell transplantation (TACT) trial published in 2002 [6]. Since then many clinical trials have been performed using various types of cells that are capable of promoting ulcer healing and neovascularization in animal models.

11.1 Endothelial Progenitor Cells

The formation of new blood vessels in response to tissue injury or ischemia is a complex physiological process. Postnatal angiogenesis was once thought to be exclusively mediated by sprouting of endothelial cells from pre-existing blood vessels. Now endothelial progenitor cells (EPCs) are well recognized to participate in new vessel formation [7]. EPCs belong to an immature cell population that is capable of differentiating into mature endothelial cells. EPCs can be isolated as $CD34^+$ or $AC133^+$ mononuclear cells (MNCs) from bone marrow or peripheral blood. Tissue ischemia or systemic administration of G-CSF, GM-CSF, vascular endothelial growth factor, or estrogen mobilizes EPCs from bone marrow into peripheral blood and the mobilized EPCs specifically home to sites of nascent neovascularization, thereby contributing to vascular repair.

The number and function of EPCs are crucial for endothelial function, especially in chronic ischemic diseases, including TAO [8–10]. Katsuki et al. found that the numbers of circulating EPCs were similar between the healthy controls and TAO patients, although the number of early outgrowth EPCs was significantly decreased in TAO patients [8]. On the contrary, Park et al. showed the number of EPCs and EPC colonies in TAO patients was significantly lower compared to the healthy control [9]. Although both teams used the same isolation and culture methods for EPCs, there was discrepancy in terms of circulating EPCs and colony-forming units in TAO patients in their reports. This discrepancy may be due to the fact that they used different definition for EPCs. Katsuki defined EPCs as $CD34^+/KDR^+$ or $CD133^+/KDR^+$ MNCs. Park et al. counted all of the cultured cells as EPCs, which were positive for vWF and VE cadherin (51% and 47%, respectively) and negative for CD34 and CD133. Idel et al. used a somewhat different method for the culture of EPCs [10]. They cultured MNCs in wells coated with human fibronectin and gelatin and maintained in endothelial cell basal medium-2 (EBM-2) supplemented with EGM-2 microvascular single aliquots and 5% fetal bovine serum. They defined ECPs as cells double-positive for lectin and Di-AcLDL. They showed the numbers of circulating EPCs were similar in the TAO group and the control group. Nevertheless, migration capacity of EPCs was not impaired in TAO patients in all of the three studies.

Yamamoto et al. quantitated mRNA expression of EPC-specific molecules (e.g., Flk-1, Flt-1, CD133, VE-cadherin, etc.) in bone marrow-derived or peripheral blood-derived MNCs obtained from four patients with ischemic limbs (two with TAO). They reported mRNA expression of EPC-specific molecules decreased in the circulating and bone marrow EPCs ($CD45^{low}CD34^+CD133^+$) [11].

In respect of the involvement of endothelial cells in the pathology of TAO, it is reasonable to propose that EPC transplantation may benefit TAO patients. As $CD133^+$ is a marker of early EPCs, Burt et al. injected $CD133^+$ stem cells collected from the peripheral blood of one TAO patient and eight other patients with critical limb ischemia to induce therapeutic angiogenesis [12]. All patients were not candidates for surgical revascularization and faced risk of amputation of the affected leg.

Stem cells were mobilized by administering G-CSF at 10 mcg/kg/day for 4–5 days. MNCs were collected by leukapheresis. CD133$^+$ stem cells were selected and injected into the patient's affected limb. Each injection delivered 2.5–5 million cells. At 12 months, two patients, both with lower extremity ulcers before treatment, underwent amputation of the treated leg. For the other seven patients, rest pain resolved within days of injection. Some functional parameters, such as treadmill pain-free walking time and treadmill exercise capacity, tended to improve but did not reach statistical significance. The authors did not describe the detailed response of the TAO patient after EPC injection.

11.2 Mononuclear Cells from Bone Marrow, Peripheral Blood, and Umbilical Cord Blood

Local injection of both bone marrow mononuclear cells (BMMNCs) and peripheral blood mononuclear cells (PBMNCs) into the ischemic limb has been proposed to initiate therapeutic angiogenesis. The strategy exploits the concept that MNCs contain EPCs that incorporate into new capillaries and other cells that will secrete factors and cytokines that may promote vessel formation.

Many studies have shown efficiency of autologous bone marrow cell injections for limb ischemia. Yamamoto et al. aspirated 400–500 mL of bone marrow from the posterior iliac crest of two TAO patients and isolated MNCs by centrifugation. Concentrated MNCs were intramuscularly injected into 40 sites of the ischemic limb. The two TAO patients were a 38-year-old woman with a painful ulcer in her left foot and a 51-year-old man with an ischemic lesion in his right foot, respectively. Ischemic status (e.g., rest pain, transcutaneous oxygen pressure, regional blood flow evaluated by thermography, and ulcer size) was dramatically improved after cell transplantation [11]. Soon Gu' team at Xuanwu Hospital reported a large clinical trial with 43 ischemic limbs in 35 patients, including 3 TAO patients. Bone marrow of each patient was stimulated by an injection of the recombinant human G-CSF (300 μg/d) for 2–3 days. In addition, heparin (12,500 units/day) was administered to avoid thrombosis. Then approximately 200 ml bone marrow (130–200 ml) was drawn from the iliac spine for MNCs. After depletion of red blood cells, concentrated BMMNCs were delivered by intramuscular injection and/or arterial intraluminal injection. Overall pain relief was remarked [13]. It is worthy to know that they administered G-CSF for 2–3 days, which may reduce cardiovascular adverse events caused by remarked increase in PBMNCs when G-CSF was administered for 5 days.

Koshikawa et al. used BMMNCs to improve symptoms of the ischemic hands in patients with peripheral arterial diseases. They enrolled six patients with TAO and one with collagen disease. Mean digital/brachial pressure index in those patients was 0.15 before transplantation and significantly increased to 0.67 at 6 months after transplantation. All patients showed improvement of pain scale and ischemic ulcers [14].

In a relatively large clinical trial, 28 homogenous TAO patients were enrolled. They were nonresponders to iloprost infusion and smoking cessation and were not candidates for nonsurgical or surgical revascularization. They all had unilateral critical limb ischemia. The patients received multiple injections of BMMNC [15]. The mean follow-up time was 16.6 months (range, 7.6–33.8 months). Only one patient required toe amputation during follow-up. A change in the ankle-brachial pressure index >0.15 was achieved in 8 patients at 3 months and in 14 patients at 6 months compared with baseline values. Patients demonstrated a significant improvement in rest pain scores, peak walking time, and quality of life. Total healing of the most important lesion was achieved in 15 patients (83%) with ischemic ulcers. Most importantly, digital subtraction angiography studies showed vascular collateral networks had formed across the affected arteries in 22 patients (78.5%).

There was another clinical trial with a large sample size that evaluated autologous BMMNC transplantation in 36 nonreconstructible TAO patients [16]. All of the patients were deemed having limb-threatening ischemia. Bone marrow was aspirated from the posterior iliac crests. The marrow was depleted of red blood cells and injected into the calf muscles of the affected limbs. At 6 months, three patients (12%) underwent major amputation. Significant improvement in skin ulcers, mean ankle-brachial index, and mean transcutaneous oximetry was observed.

The long-term effect of BMMNCs for TAO was also excellent in a study with eight TAO patients [17]. Eleven limbs (3 with rest pain and 8 with an ischemic ulcer) of 8 TAO patients were followed up for clinical events for a mean of 684 ± 549 days (range 103–1466 days) after BMMNC transplantation. At 4 weeks, improvement in pain was observed in all 11 limbs, with complete relief in 4 (36%). VAS pain score decreased from 5.1 ± 0.7 to 1.5 ± 1.3. An improvement in skin ulcers was observed in all eight limbs with an ischemic ulcer, with complete healing in seven (88%).

In the 3-year-follow-up study of TACT trial [6], which included 115 patients (74 with peripheral arterial disease and 41 with TAO), 3-year mortality and leg amputation-free interval were accessed as primary end points [18]. The median follow-up time for surviving patients was 25.3 months. In the TAO subgroup, the 3-year overall survival rate was 100% (no death), and the 3-year amputation-free rate was 91%. The significant improvement in the leg pain scale, ulcer size, and pain-free walking distance was maintained for at least 2 years after the therapy. They concluded that the safety and efficacy of BMMNC transplantation were not inferior to the conventional revascularization therapies.

Most recently, Gu's team reported their 10-year follow-up of TAO patients after BMMNC transplantation [19]. This is so far the longest follow-up report of the similar studies, and the results confirmed the safety and efficacy of BMMNCs for TAO. During January 2005 and July 2006, 59 patients with TAO were treated with either aspirin alone ($n = 19$) or aspirin plus BMMNC injection ($n = 40$). Concentrated ABMMNCs were injected at 5 cm proximal to the obstructive lesion (Fig. 11.2). The 10-year amputation-free survival was 85.3% (29/34) in patients treated with BMMNCs compared to 40% (6/15) in patients treated with aspirin alone. Ulcer
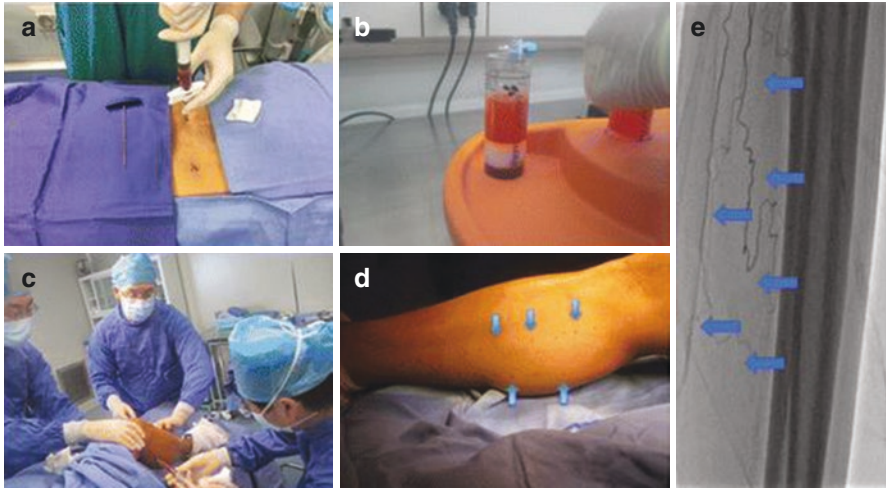
Fig. 11.2 Representative photos of BMMNC treatment. (**a**) Bone marrow was harvested from the posterior superior iliac spine. (**b**) Separation of red cells and BMMNCs. (**c**) BMMNC injection. (**d**) Injection sites. (**e**) Representative angiogram. Arrows indicate injection sites along the tibial arteries

area, toe-brachial index, transcutaneous oxygen pressure, and pain score were also significantly improved with BMMNC treatment (Fig. 11.3).

Another long-term follow-up study by Baran et al. also supported the benefit of autologous BMMNC transplantation in TAO patients, even though this study had a smaller sample size [20]. Twenty-eight patients (25 males and 3 females) were enrolled between April 2003 and August 2005. BMMNCs were injected into the gastrocnemius muscle, the intermetatarsal region, and the dorsum of the foot ($n = 26$) or forearm ($n = 2$), and saline was injected into the contralateral limbs. The mean follow-up time was 139.6 ± 10.5 months. The ankle-brachial pressure index evaluated at 6 months and 120 months was improved compared to the baseline scores. The angiographic improvement was 78.5% and 57.1% at 6 and 120 months, respectively. The 10-year amputation-free rate was 96% in BMMNC-implanted limbs and 93% in saline-injected limbs. The high amputation-free rate in the saline-injected limbs was interesting. We think this is because BMMNCs secrete many proangiogenic factors into the peripheral circulation and exert proangiogenic effect in the distal saline-injected limbs.

Kim et al. achieved autologous whole bone marrow stem cell transplantation via fenestration of the tibia bone [21]. Twenty-seven TAO patients (34 lower limbs) who were not candidates for surgical revascularization or radiologic intervention were enrolled. Fenestration through the tibia bone was performed under general or spinal anesthesia. Six tibial sites were fenestrated with a 2.5-mm-diameter screw under fluoroscopic guidance. This procedure allowed bone marrow stem cells to be released into ischemic calf muscle through fenestrated posterior tibia holes. The mean follow-up time was 19.1 months. Before treatment all of the 34 limbs had symptoms

Fig. 11.3 Representative photographs showing the gradual healing of an ulcer during the follow-up period. (**a**) Before BMMNC transplantation. (**b**) Three months after BMMNC transplantation. (**c**) Six months after BMMNC transplantation. (**d**) Ten years after BMMNC transplantation

that were more than category 3 (severe claudication). During the follow-up period, 23 limbs (67.6%) had symptoms of category 2 (moderate claudication). One limb achieved a +3 (markedly improved) outcome, 26 (76.5%) achieved a +2 (moderately improved) outcome, and 7 (20.6%) limbs were unchanged. Thirteen of 17 limbs with nonhealing ulcer healed during follow-up. Although the authors concluded this method was effective in inducing therapeutic angiogenesis in TAO patients, we think it is more invasive compared to intramuscular injection of BMMNCs.

Although MNC transplantation for ischemic limb disease was first performed with BMMNCs, PBMNCs are also widely used. PBMNCs can be harvested in an easier and less invasive approach. The mononuclear cell fraction is usually enriched by Ficoll density gradient system centrifugation and by use of blood centrifugation and plasmapheresis systems after G-CSF or GM-CSF mobilization. Moriya et al. retrospectively evaluated 14 TAO patients who were treated between July 2002 and December 2005 and analyzed the data in December 2007. Improvement of ischemic symptoms was remarked. Only one TAO patient underwent major amputation during the observation period [22]. Horie et al. reported excellent long-term clinical outcomes for patients with lower limb ischemia implanted with G-CSF-mobilized autologous PBMNCs [23]. Among 11 patients with TAO, the 2-year survival rate

was 100%; the 1-year amputation-free rates were 79%. Ishida et al. enrolled five patients with TAO and one patient with arteriosclerosis obliterans. PBMNCs were harvested and injected intramuscularly (five legs and one arm) for 2 days. Improvement in the ankle-brachial pressure index was seen in four patients at 4 weeks, and ischemic ulcer improved in three patients at 3 weeks. The mean maximum walking distance significantly increased from 203 m to 559 m at 4 weeks and was sustained for 24 weeks [24].

Furthermore, Kim AK et al. used human umbilical cord blood-derived mononuclear cells (UCBMNCs) for TAO [25] after they had tried BMMSCs [21]. UCBMNCs should be associated with less graft-versus-host disease, and the human leucocyte antigen (HLA) matching should be less stringent because of the immunological naiveté of UCBMNCs. In their study, seven TAO patients (seven lower limbs) who had intermittent claudication, rest pain, and foot ulcer for a minimum of 3 months without evidence of improvement with medical treatment were enrolled. The patients continued their medications during the study period. A total of 4×10^8 ABO type-matched HCBMNCs were injected into 20 sites of the ischemic calf muscles along the tibial and peroneal arteries. After the procedures, the patients were followed up at 1, 3, 6, and 9 months. The patients did not receive any immunosuppressive drugs and did not experience any graft-versus-host disease during the follow-up period. However, the authors did not report specifically the efficacy of HCBMNC transplantation in the TAO patients [25].

We notice that most of the studies using MNCs for TAO did not observe any serious adverse events. Surprisingly, one patient suffered sudden death at 20 months after transplantation of BMMNCs, and the authors thought it was associated with injection of BMMNCs [14]. The sudden death happened when the patient was 30 years old. He had no risk factors for atherosclerosis and stopped smoking before BMMNC injection. Furthermore, [201]thallium scintigraphy performed before injection revealed no sign of myocardial ischemia. Considering the patient's background and the natural course of TAO, the authors thought that his death was related to BMMNC transplantation. Indeed, several studies have suggested the possible role of BMMNCs in atherogenesis. Silvestre et al., for example, demonstrated that transplantation of BMMNCs into ischemic limbs of apolipoprotein E-knockout mice led to a significant increase in atherosclerotic plaque size in the aortic sinus despite the fact that the total cholesterol levels were within normal range and there was no significant change in plaque stability [26]. Therefore they suggested transplantation of BMMNCs may contribute to "silent" progression of atherosclerosis, which could be harmful in the long term. More recently, George et al. have also shown that an intravenous injection of bone marrow cells into apolipoprotein E-knockout mice resulted in an increase in atherosclerotic lesion size, whereas an injection of EPCs influenced plaque stability [27]. We should note that in the experiments with apolipoprotein E-knockout mice, BMMNCs were intravenously injected, whereas in the human clinical trials, BMMNCs were injected locally in the affected legs. In fact, three long-term follow-ups did not observe similar adverse events [18–20]. Nevertheless, more patients are needed to establish a definite relationship between BMMNC transplantation and atherosclerosis.

11.3 Hematopoietic Stem Cell

Hematopoietic stem cells (HSCs) play important roles in angiogenesis. In a study by Kinoshita et al., 15 TAO patients received intramuscular injection of G-CSF mobilized CD34+ cells from peripheral blood [28]. PBMNCs were harvested after bone marrow mobilization with G-CSF (10 mg/kg per day) for 5 days. Leukapheresis was performed to harvest PBMNCs, and magnetic separation was used for CD34+ cells. Purified CD34+ cells were intramuscularly injected into 40 sites (30 sites in the calf, 6 sites in the sole, and 4 sites in the intertoe muscle) of the leg. Dose of CD34+ cells was 10^5 cells/kg, 5×10^5 cells/kg, or 10^6 cells/kg. Favorable results were observed. Rutherford scale significantly improved by week 8, and the improvement sustained until week 208. CLI-free ratio increased and peaked (100%) at week 156. Due to the high cost of magnetic separation for purification of CD34+ cells, we think HSCs are not preferred cells for the treatment of TAO unless HSCs are proved to be superior to other types of cells in terms of efficacy and safety.

11.4 Mesenchymal Stem Cells

As we have mentioned in the other chapter, mesenchymal stem cells (MSCs) were originally isolated from bone marrow and later from a variety of tissues, such as adipose tissue, tooth pulps, periodontal tissue, umbilical cord, and placenta. MSCs from umbilical cord, adipose tissue, and bone marrow have been used for TAO. The invasive procedure for harvesting autologous MSCs from bone marrow or adipose tissue is a disadvantage.

MSCs are well studied for their ability to promote angiogenesis and arteriogenesis through stromal and paracrine activity. Theoretically, UCBMSCs have many advantages because of abundant resource and the putative stronger stemness of newborn cells compared with adult stem cells. Cell therapy using UCBMSCs also have the merit of being less invasive and less expensive because it is easy to mass-produce UCBMSCs as a commercial product.

Interestingly, in vitro experiment evaluating the promoting effects of MSCs on human umbilical vein endothelial cell proliferation, migration, and tube formation and in vivo experiment evaluating the effects of MSCs in animal models of hindlimb ischemia showed that UCMSCs were superior to BMMSCs and adipose tissue-derived MSCs (ATMSCs) were superior to UCMSCs [29, 30].

Dash et al. first conducted a randomized controlled trial using BMMSCs for chronic nonhealing ulcers of the lower extremities. A total of 24 patients, 6 with diabetic foot ulcers and 18 with TAO, were enrolled and randomized into implant and control groups. In the implant group, the patients received autologous cultured BMMSCs along with standard wound dressing; the control group received only the standard wound dressing regimen. They assessed ulcer size at the beginning of the study and 12 weeks after treatment. A larger decrease in ulcer area in the BMMSC

group was observed: mean ulcer area decreased from 5.04 cm^2 to 1.48 cm^2. In the control group, the mean ulcer area decreases from 4.68 cm^2 to 3.59 cm^2. Pain-free walking distance increased from 38.33 meters to 284.44 meters in the BMMSC group. On the contrary, mean pain-free walking distance increased from 35.66 meters to 78.22 meters in the control group [31].

A prospective, double-blind randomized placebo-controlled multicenter study was conducted by Gupta et al. that used allogeneic BMMSCs to patients with established arterial occlusive disease (Rutherford II-4, III-5, or III-6) and was not suitable for or had failed revascularization treatment. Twenty patients (BMMSC: placebo = 1:1) were administered with at a dose of 2 million cells/kg or placebo (PlasmaLyte A) at the gastrocnemius muscle of the ischemic limbs. Significant increase in ankle-brachial pressure index and ankle pressure was seen in the BMMSC group compared to the placebo group. However, the authors did not reported how many TAO were included in their cohort [32]. They further conducted a prospective, dose-finding phase II study assessing the efficacy and safety of intramuscular administration of allogeneic BMMSCs in TAO patients. Cells were injected intramuscularly in the gastrocnemius muscle and locally around the nonhealing ulcer. Significant reduction of rest pain and healing of ulcers in an accelerated fashion were observed [33].

Kim reported for the first time a clinical trial on TAO patients using UCBMSCs [34]. They first proved that transplantation of UCBMSCs augmented arteriogenesis in the ischemic limb of immunodeficient nude mice. Then four TAO patients were enrolled for UCBMSC transplantation. All patients had already received medical treatment, surgical interventions, and even amputations, but they had to take painkillers to sleep at night. The human leukocyte antigen (HLA) typings were done to get a proper match between the patients and preserved umbilical cord blood. UCBMSCs (1×10^6) were injected into the adjacent area of the lesions with a 23-gauge needle syringe. Strikingly, the pain of the patients was alleviated more rapidly than the formation of the new capillaries, which happened between 5 hours and 14 days in all patients. The unhealed skin lesions of the two patients showed skin regeneration within 120 days. Allograft rejection was not observed, and even no immunosuppressants were administered during the follow-up periods of up to 25 months in the first enrolled patient.

In another phase I study with 8 patients (5 with TAO), Yang et al. injected a total of 1×10^7 UCBMSCs into 20 sites on each limb using 23-gauge needle. Injection sites were selected below the knee at 20 different sites of ischemic calf muscle along the tibial and peroneal arteries [35]. During the 6-month follow-up period, no death or serious adverse events were observed, and there were no amputations. Angiography revealed increasing scores compared with those at baseline in three of eight patients. Improvement in ulcer healing and an increase in pain-free walking distance were also observed.

Several groups used ATMSCs for proangiogenic therapy. In a phase I trial the safety and efficacy of autologous cultured ATMSCs to treat patients with non-revascularizable critical limb ischemia were evaluated. There was one male TAO patient with Rutherford grade III-6. Adipose tissues were obtained by simple liposuction from the abdominal subcutaneous fats and digested with collagenase I. Patients

were treated with ATMSCs by intramuscular injection into the ischemic leg. Rest pain and TcPO2 all improved during the 6-month follow-up [36].

In the study by Lee et al., 12 TAO patients and 3 patients with diabetic foot were treated with intramuscular injections of ATMSCs [37]. A total of 3×10^8 ATMSCs (each syringe had 5×10^6 ATMSCs) were injected at 60 points into the lower extremities of 12 patients. Clinical improvement occurred in 10 patients (66.7%), including 7 of 12 TAO patients (58.3%). Four TAO patients required minor amputation during follow-up, and all amputation sites healed completely. Digital subtraction angiography before and 6 months after ATMSC implantation showed formation of numerous vascular collateral networks across affected arteries. Improvement of more than one grade in collateral vessel formation was observed in two of the three patients with diabetic foot (66.7%) and in eight of the ten patients with TAO (80%). A two-year long studies with ATMSCs in 17 TAO patients also showed the onetime ATMSC therapy can provide long-term benefit to patients as indicated by decreased rest pain, increased pain-free walking distance, increased toe-brachial pressure index, increased transcutaneous oxygen pressure, and increased arterial brachial pressure index and freedom from amputations during the follow-up [38].

11.5 Conclusions

TAO represents a most severe disease that profoundly influences patient quality of life. Prompt recognition, medication, and revascularization are the current standards of care. Nevertheless, this care strategy is not completely effective. Researches for new pharmacological and angiogenic therapies are underway to meet this unmet medical need. Current literature supports that intramuscular administration of BMMNCs, PBMNCs, and ATMSCs is a relatively safe and effective therapy for TAO patients not suitable to conventional revascularization. Adverse events are mostly mild and are related to local implantation/injection. Although encouraging results from multiple studies have been reported, unfortunately such studies involved small numbers of patients. There is a need for larger, placebo-controlled, randomized multicenter trials to confirm safety and efficacy of these cell therapies.

Animal studies indicate that paracrine, anti-apoptotic growth factor, or other factors produced by stem cells may mediate the response independent of direct differentiation into endothelial cells. However, the precise molecular mechanisms of stem cell therapy are still unknown. The specific mechanisms for the improvement in the patients' pain remain to be fully explained because the quick reduction of rest pain was noticed before vessel formation. In addition, many questions remain unanswered with regard to stem cell therapy, including identification of the ideal cell types, autologous versus allogeneic cells, optimal cell number, and route and frequency of administration. Effective protocols regarding in vitro cell methods for augmentation of cell potency, which may include stimulation of the stem cells with small molecule, cytokines, and various growth factors and use of bioactive microspheres, are also needed to be explored and evaluated in clinical trials.

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