

## Review Article

# Impact of Therapeutic Angiogenesis Using Autologous Bone Marrow-derived Mononuclear Cell Implantation in Patients with No-option Critical Limb Ischemia

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Recently, the limb salvage rate of patients with critical limb ischemia (CLI) has been improved due to the development of revascularization and wound care treatment. However, many patients with CLI are refractory to standard treatments, including revascularization such as endovascular treatment or surgical bypass. Establishment of a new cell therapy is required to improve the limb salvage rate and prognosis in patients with CLI. In 1997, endothelial progenitor cells were found to be derived from the bone marrow to circulate as CD34 surface antigen positive cells in peripheral blood and to affect therapeutic angiogenesis in ischemic tissues. Later, therapeutic angiogenesis using autologous bone marrow-derived mononuclear cell (BM-MNC) implantation was performed for patients with no-option CLI in clinical practice. Several reports showed the safety and efficacy of the BM-MNC implantation in patients with CLI caused by arteriosclerosis obliterans, thromboangiitis obliterans (TAO), and collagen diseases. In particular, in patients with CLI caused by TAO, limb salvage rate was significantly improved compared with standard treatments. The BM-MNC implantation may be feasible and safe in patients with no-option CLI. Here, we review the efficacy of BM-MNC implantation in no-option CLI, with a focus on therapeutic angiogenesis.

**Keywords:** critical limb ischemia, therapeutic angiogenesis, bone marrow-derived mononuclear cell, salvage rate, no-option case

## Introduction


Critical limb ischemia (CLI) is a disease that causes rest pain, ulcers, and gangrenes owing to tissue ischemia induced by arteriosclerosis and vascular occlusion. CLI can be caused by arteriosclerosis obliterans (ASO), thromboangiitis obliterans (TAO), and collagen diseases (CD). Patients with CLI may require limb amputation due to unbearable pain and the risk of infection, leading to reduced activities of daily living and quality of life. In particular, in patients with CLI caused by ASO, it was reported that the current annual incidence of limb amputation is approximately 25%, and the 5-year survival rate is approximately 50%.<sup>1,2)</sup>

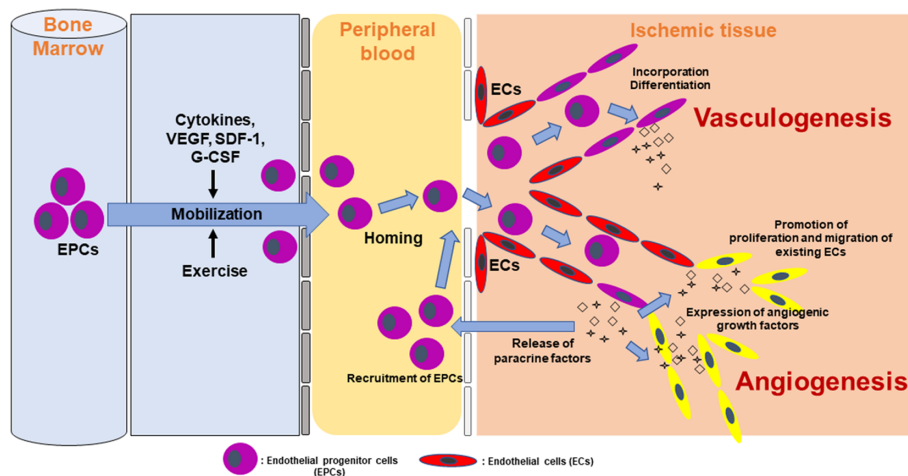
Currently, revascularization therapies such as endovascular treatment (EVT) or bypass surgery are administered as standard treatments for patients with CLI in addition to drug therapy and cessation of smoking. However, it reported a high restenosis and revascularization rate after balloon angioplasty due to severe calcification in many patients with ASO, particularly those patients with dialysis. In addition, poor outcomes of EVT and bypass surgery have been recently reported in patients with narrowing of the peripheral blood vessels below the ankle and patients with severe runoff vessel diseases.<sup>3)</sup> In patients with TAO or CD, poor long-term patency rate after bypass surgery has been also reported.<sup>4-6)</sup> In many patients with TAO or CD, bypass surgery is difficult because the peripheral arteries are markedly narrowed. Furthermore, there is no sufficient evidence that shows the benefits of EVT because they often exhibit early recoil or reocclusion owing to vascular properties.

In the future, the number of no-option CLI patients (CLI patients who cannot undergo revascularization or

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**Fig. 1** Kinetics of the endothelial progenitor cells (EPCs) for post-natal neovascularization. EPCs: endothelial progenitor cells; ECs: endothelial cells; VEGF: vascular endothelial growth factor; SDF-1: stromal-derived factor; G-CSF: granulocyte colony-stimulating factor.

CLI patients who are refractory to standard treatments) is expected to increase. So, in patients with no-option CLI, new cell therapy is required to increase the limb salvage rate and improve their prognosis.

In 1997, endothelial progenitor cells (EPCs) derived from the bone marrow were reported to promote angiogenesis.<sup>7,8)</sup> Later, many clinical trials have reported the safety and efficacy of therapeutic angiogenesis using autologous bone marrow-derived mononuclear cell (BM-MNC) implantation in patients with no-option CLI. In this review, we discuss the BM-MNC implantation in patients with no-option CLI.

## Mechanism of Neovascularization

The conventional mechanism of neovascularization in adults is mainly based on the concept of angiogenesis in which blood vessels develop through the proliferation and migration of preexisting endothelial cells.<sup>9)</sup> However, in 1997, Asahara et al. found that CD34 surface antigen positive (CD34+) cells isolated from peripheral blood mononuclear cells (PBMCs) are able to differentiate into endothelial cells.<sup>7)</sup> In addition, CD34+ cells were found to express vascular endothelial cell markers, such as CD31, Flk-1, and Tie-2, and to express other markers, i.e., Flt-1 and Tie-1, after cell differentiation. Moreover, after systemic transplantation of CD34+ cells into mice with lower-limb ischemia, CD34+ cells were incorporated into ischemic lesions and contributed to the differentiation and migration of preexisting endothelial cells, leading to angiogenesis. In a mouse model of bone marrow transplantation using transgenic mice that express  $\beta$ -galactosidase under the control of Flk-1 and Tie-2, which are specifically expressed in vascular endothelial cells, researchers

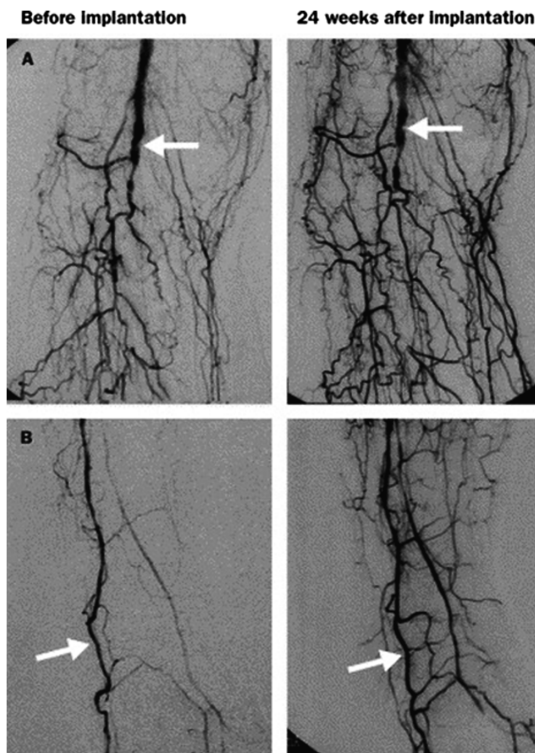
confirmed that cells expressing bone marrow-derived Flk-1- or Tie-2-expressing cells were incorporated into the neovasculature after induction of ischemia.<sup>8)</sup> Based on these findings, EPCs were found to be derived from the bone marrow to circulate as CD34+ cells in peripheral blood and to be involved in neovascularization. The results showed that vasculogenesis occurs not only in the embryonic period but also in adulthood.

During tissue ischemia, EPCs are mobilized from the bone marrow by cytokines and growth factors (e.g., vascular endothelial growth factor [VEGF], stromal-derived factor [SDF]-1, granulocyte colony-stimulating factor [G-CSF], angiopoietin-1, estrogen, and erythropoietin), leading to increases in the numbers of EPCs in peripheral blood.<sup>10-15)</sup> In addition, external factors, such as exercise, also contribute to an increase in the number of EPCs.<sup>16)</sup> The mobilized EPCs were recruited to ischemic tissue, and the accumulation of EPCs directly contribute to vasculogenesis by providing components of the blood vessel wall. Furthermore, EPCs synthesize and secrete various cytokines (e.g., VEGF, insulin-like growth factor-1, SDF-1, hepatocyte growth factor, and G-CSF), thereby promoting the proliferation and migration of existing endothelial cells.<sup>17)</sup>

These basic experiments demonstrated that bone marrow-derived EPCs are involved in neovascularization in the ischemic penumbra by angiogenesis and vasculogenesis (Fig. 1). Therefore, EPCs may have applications in the treatment of patients with CLI.

## First Clinical Trials of Therapeutic Angiogenesis Using Autologous BM-MNC Implantation in Patients with CLI

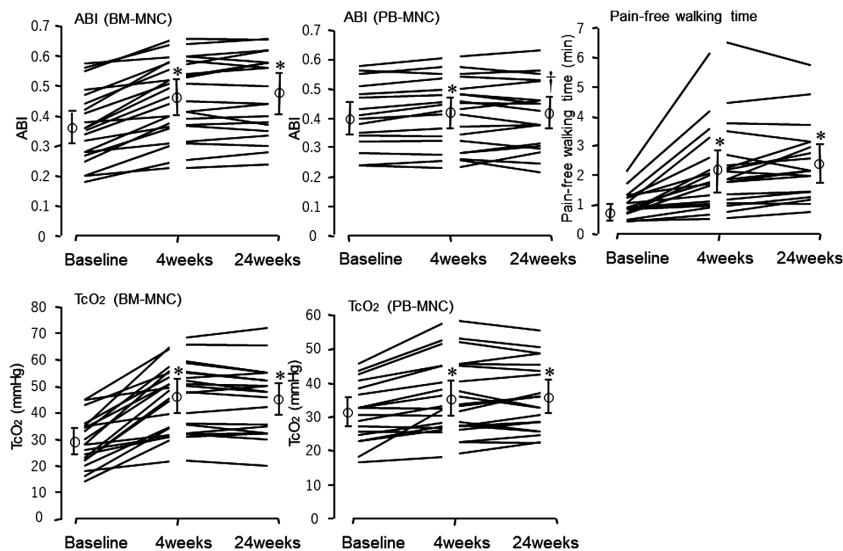
Tateishi-Yuyama et al. reported an initial randomized,



**Fig. 2** Angiographic analysis of collateral vessel formation before and after the bone marrow-derived mononuclear cell (BM-MNC) implantation.

Analysis of collateral branches in the (A) knee and upper tibia and in the (B) lower tibia ankle and foot, 24 weeks after the BM-MNC implantation compared with before the BM-MNC implantation. Contrast densities in posterior-tibial and dorsal pedal arteries are shown (arrows). Reprinted with permission from Tateishi-Yuyama et al., *Lancet* 2002.

clinical pilot study for angiogenic cell therapy using intramuscular injection of autologous BM-MNCs into 45 patients with peripheral artery disease (PAD) with critically ischemic legs (Japan Trial for Therapeutic Angiogenesis using Cell Transplantation [J-TACT]) in 2002.<sup>18)</sup> In this study, 29 patients with unilateral limb ischemia were recruited, 25 of whom were transplanted with BM-MNCs into the gastrocnemius of the ischemic limb (ankle-brachial index [ABI]<0.6). Saline was injected as a control treatment into the opposite, less ischemic leg (ABI >0.6). In addition, 22 patients with bilateral leg ischemia were also recruited, and BM-MNCs (as an active treatment) or PBMCs (as a control treatment) were randomly injected into their ischemic legs. Overall, the implantation of autologous BM-MNCs was found to be safe and effective. Angiography results demonstrated a marked increase in the number of visible collateral vessels (Fig. 2). Significant increases in pain-free walking time, rest pain, and tissue oxygen pressure were observed 6 months after treatment, whereas injection of PBMCs (as a control) yielded less significant effects (Fig. 3). Because BM-MNC preparations contain EPCs and can release various angiogenic factors, incorporation of EPCs into newly formed vessels and the angiogenesis induced by angiogenic factors secreted from the injected cells could contribute to increased blood flow; this novel cell therapy may be a promising new therapeutic strategy for treating patients with PAD with critically ischemic legs. Since then, the duration of the effects of therapeutic angiogenesis in patients with CLI has been validated during follow-up in clinical trials (from 3 weeks to 3 years), and many reports have been published (Fig. 4).<sup>18-28)</sup>



**Fig. 3** Improvement of efficacy measurements 4 and 24 weeks after the BM-MNC implantation. Data are expressed as means±standard deviations. ABI: ankle-brachial index; TcO<sub>2</sub>: transcutaneous oxygen pressure. Reprinted with permission from Tateishi-Yuyama et al., *Lancet* 2002.

Year	Source	Pts	No. of Pts	Total Cell Number (x10 <sup>9</sup> )(SD)	CD34+ Number (x10 <sup>4</sup> )(SD)	Outcome	Follow-up period
2002	Tateishi-Yuyama et al, <sup>18</sup>	CLI (unilateral) (bilateral)	Total 45 25 20	1.6(0.6) 1.5(0.6)	3.9(2.2) 3.5(1.3)	Significant improvement in ABI, TcO <sub>2</sub> , VAS, walking time, angiography Significant improvement in ABI, TcO <sub>2</sub> , VAS, walking time	4Wks, 24Wks
2004	Miyamoto et al, <sup>19</sup>	CLI	12	4.0(0.3)	1.9(0.3)	Significant improvement in ABI, VAS, walking time, Tc-TF perfusion scintigraphy	4Wks,
2006	Bartsch et al, <sup>20</sup>	PAD	10	0.1(-)	-	Significant improvement in ABI, walking distance	8Wks
2006	Durdu et al, <sup>21</sup>	TAO	28	1.7(0.9)	5.3(3.6)	Significant improvement in VAS, walking time, ulcer size, angiography, QOL Improvement in ABI	3M, 6M
2006	Barc et al, <sup>22</sup>	CLI	15	-	-	Marginally improved in ulcer size No improvement in ABI, TcO <sub>2</sub> , angiography	(1M) 3M,(6M)
2006	Gu et al, <sup>23</sup>	CLI	35	5.6	-	Significant improvement in ulcer size, VAS,ABI, TcO <sub>2</sub> ,	2M
2007	Bartsch et al, <sup>24</sup>	CLI	13	0.1(0.03)	-	Significant improvement in ABI, walking distance, Capillary-venous SaO <sub>2</sub>	2M,4M 13M
2007	Oda et al, <sup>25</sup>	CLI	21	3.0(1.4)	6.7(7.6)	Significant improvement in ABI, TcO <sub>2</sub> ,	4Wks
2007	Saito et al, <sup>26</sup>	CLI (TAO)	14	4.2(4.0)	-	Significant improvement in VAS, ulcer size, angiography,	4Wks, 24Wks
2008	Gan et al, <sup>27</sup>	CLI	15	0.2-0.9	0.9-11.0	Significant improvement in ABI, walking distance	12wks
2008	Matoba et al, <sup>28</sup>	CLI PAD TAO	Total 115 (74) (41)	3.1(2.8) 4.1(3.6)	2.6(2.3) 4.6(4.0)	Significant improvement in VAS, walking time, ulcer size No improvement in ABI,TcO <sub>2</sub>	3 years

Fig. 4 Clinical reports after the BM-MNC implantation.

CLI: critical limb ischemia; PAD: atherosclerotic peripheral artery disease; TAO: thromboangiitis obliterans; ABI: ankle-brachial index; TcO<sub>2</sub>: transcutaneous oxygen pressure; VAS: visual analog scale; SaO<sub>2</sub>: oxygen saturation.

## Safety and Effectiveness of Therapeutic Angiogenesis Using Autologous BM-MNC Implantation

### Results in patients with ASO and TAO

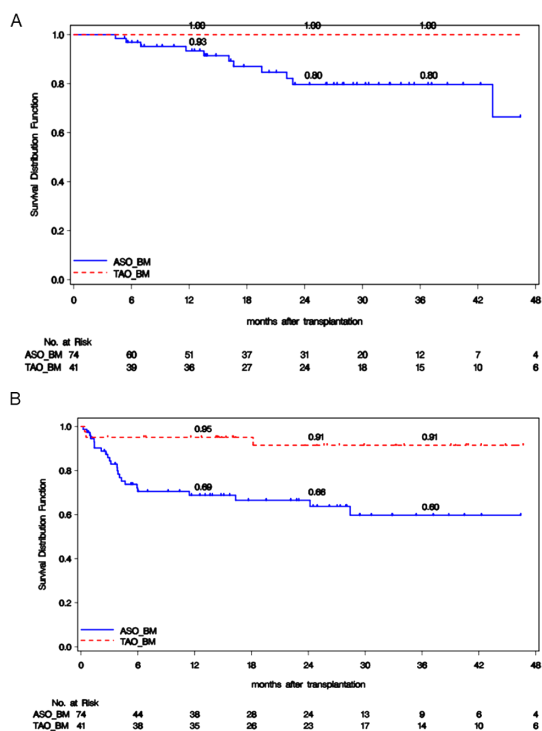
In the world's first multicenter study (J-TACT), BM-MNC implantation was performed in 45 patients with lower-limb ischemia (Fontaine classification stages III and IV) who showed no improvement after surgical or standard nonsurgical treatment. After treatment, 18 of 20 patients exhibited complete pain relief in the lower limbs. Treadmill walking distance until patients felt pain increased by approximately 2.6 times. Follow-up angiography showed a significant increase in collateral circulation in 27 of 45 patients. After these results were reported, the number of participating centers increased to 11, and the number of enrolled patients increased to 115 (74 patients with ASO and 41 patients with TAO). Figure 5 shows overall survival and limb salvage rates at 3 years after the BM-MNC implantation. The overall survival rates were 80% for the ASO group and 100% for the TAO group. During the 3-year follow-up, 11 of 74 patients with ASO died, whereas no patients with TAO died. In addition, some patients in the ASO group had severe adverse events, whereas only one patient in the TAO group had severe adverse events. However, no patients died of adverse events related to the BM-MNC implantation. The 3-year limb

salvage rates were 60% for the ASO group and 91% for the TAO group.<sup>28)</sup>

Figure 6 shows the endpoints after the BM-MNC implantation. There were no significant improvements in ABI in the ASO and TAO groups. However, the results showed that rest pain (evaluated by visual analog scale [VAS]) was reduced, walking distance (the distance until lower extremity pain occurred) was increased, and the diameter of the ulcer (cm<sup>2</sup>) decreased. In most patients, these improvements were observed within 6 months after the BM-MNC implantation, and the effects persisted.<sup>28)</sup>

Subsequently, a randomized trial of therapeutic angiogenesis using autologous BM-MNC implantation in patients with ASO and TAO versus a control group receiving standard treatment was reported in 2011. During the 4-year follow-up in the ASO group, the limb salvage rate was 48% for the therapeutic angiogenesis group and 0% for the control group. The overall survival rates in the therapeutic angiogenesis and control groups were 76% and 67%, respectively. Although not statistically significant, therapeutic angiogenesis increased the limb salvage rate and overall survival in the ASO group. On the other hand, during the 4-year follow-up in the TAO group, the salvage rate was 95% for the therapeutic angiogenesis group and 6% for the control group. The overall survival rate was 100% in both groups. The therapeutic angiogenesis using autologous BM-MNC implantation has been





**Fig. 5** Three-year overall survival and major amputation-free rates after the BM-MNC implantation in patients with atherosclerotic peripheral artery disease (PAD) or TAO.

**A)** Three-year overall survival rates in 74 patients with atherosclerotic PAD and 41 patients with TAO. **B)** Three-year major amputation-free rates in patients with atherosclerotic PAD and patients with TAO. CI: confidence interval. Reprinted with permission from Matoba et al., *Am Heart J* 2008.

reported to significantly increase the limb salvage rate in patients with TAO.<sup>29)</sup>

### Results in patients with CD

The overall survival and limb salvage rates at 1 year after the BM-MNC implantation in all patients with CD were 98% and 95%, respectively (Fig. 7).<sup>30,31)</sup> In this study, patients with CD were divided into scleroderma (SSc) and non-SSc groups according to the immunological mechanism for each disease. The SSc group included some SSc-related diseases (SSc; calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; and mixed connective tissue disease). The non-SSc group included other CDs (systemic lupus erythematosus, rheumatoid arthritis, polyarteritis nodosa, antiphospholipid syndrome, Sjögren's syndrome, eosinophilic granulomatosis with polyangiitis, Behçet's disease, and vasculitis without a definite diagnosis). Figure 8 shows the alleviation of rest pain within 6 months after the BM-MNC implantation in patients with CD (the SSc and non-SSc groups). Both groups showed significant reductions in rest pain within 6 months after the BM-MNC implantation. Addi-

tionally, increased overall survival and limb salvage rates (particularly in the SSc group) were observed at 1 year after the BM-MNC implantation (Fig. 9).<sup>30)</sup>

These results suggest that the BM-MNC implantation is safe and effective in patients with no-option CLI. In particular, the BM-MNC implantation is effective at alleviating ischemic symptoms in patients with TAO and CD. However, several factors, such as maintenance dialysis, history of lower-limb bypass surgery, and the severity of ischemia affect prognosis and limb salvage rates in patients with no-option CLI caused by ASO after the BM-MNC implantation.

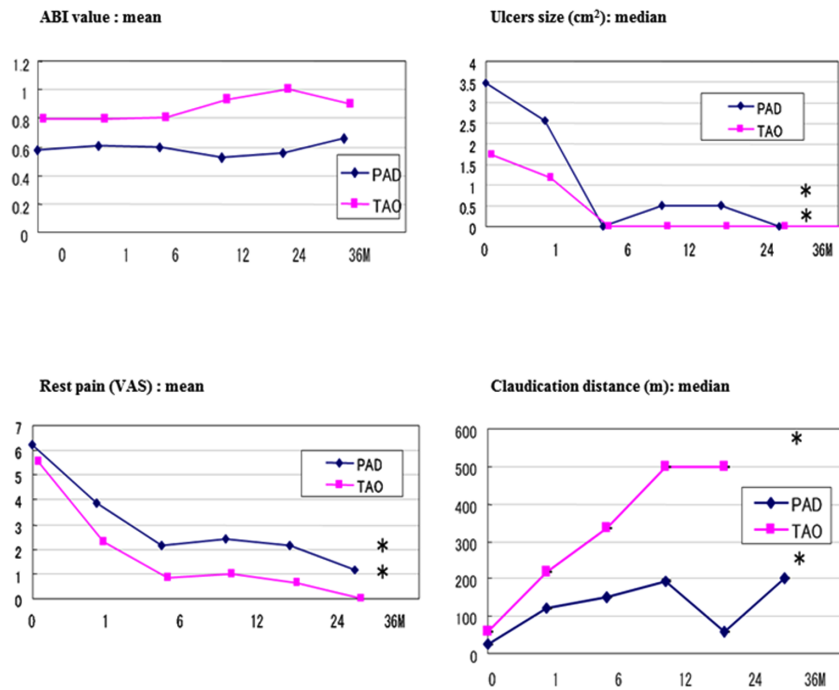
### Long-term Clinical Outcomes after Therapeutic Angiogenesis Using Autologous BM-MNC Implantation

In 2018, the J-TACT study of long-term prognosis was reported.<sup>31)</sup> The 5- and 10-year overall survival rates of all patients with CLI were 87% and 69%, respectively. The 5- and 10-year overall survival rates in the ASO group were 75% and 47%, respectively; the 5-year overall survival rates in the TAO and CD groups were 98% and 95%, respectively; and the 5- and 10-year limb salvage rates in all patients with CLI were 82% and 81%, respectively. Additionally, the 5- and 10-year limb salvage rates in the ASO group were 74% and 70%, respectively, and the 5-year limb salvage rates in the TAO and CD groups were 88% and 91%, respectively, demonstrating long-term limb salvage (Fig. 10).<sup>31)</sup>

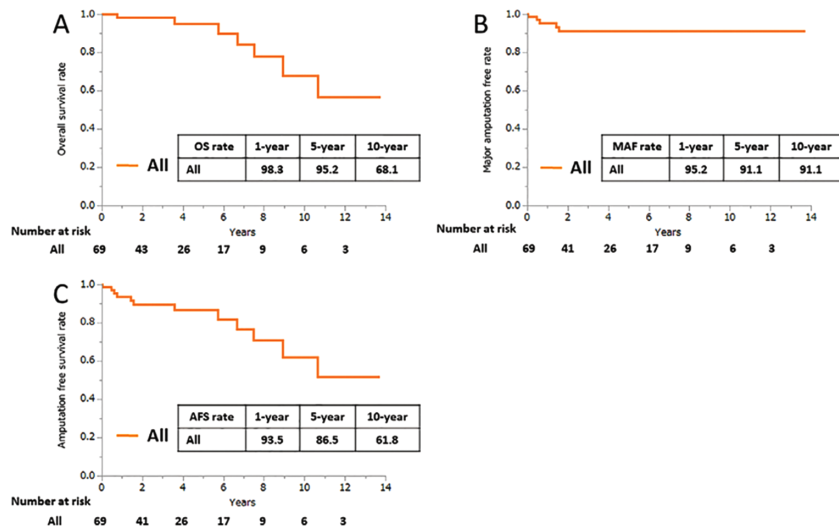
### Discussion

These results show the safety and efficacy of the BM-MNC implantation in patients with no-option CLI. In particular, patients with TAO and CD show good responses to this cell therapy.

Generally, bypass surgery and EVT are important and effective treatment methods for alleviating ischemic symptoms (promoting wound healing and reducing rest pain) in patients with CLI. Five-year limb salvage rate (83.5% versus 55.8%) and the 5-year amputation-free survival (AFS) rate (57.7% versus 36.0%) were significantly higher in the CLI group subjected to revascularization than in the CLI group without revascularization.<sup>4)</sup> However, approximately one-third of patients with CLI cannot undergo surgery for various reasons, including complications (e.g., cardiac/respiratory dysfunction), unfavorable general conditions (e.g., severe dementia, being bedridden, extensive necrosis, or infection), and technical issues (e.g., absence of an artery that can be grafted).<sup>4)</sup> Benoit et al. showed that the 1-year major AFS rate was 62%–90% and that the 1-year AFS rate was 48%–81% in 2006–2010 for



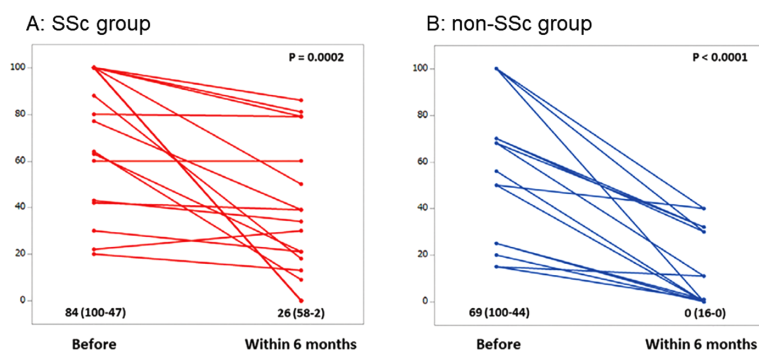
**Fig. 6** Time-dependent changes in ABI, rest pain (VAS), ulcer size, and claudication walking distance after the BM-MNC implantation. ABI: ankle-brachial index; VAS: visual analog scale. Reprinted with permission from Matoba et al., Am Heart J 2008.



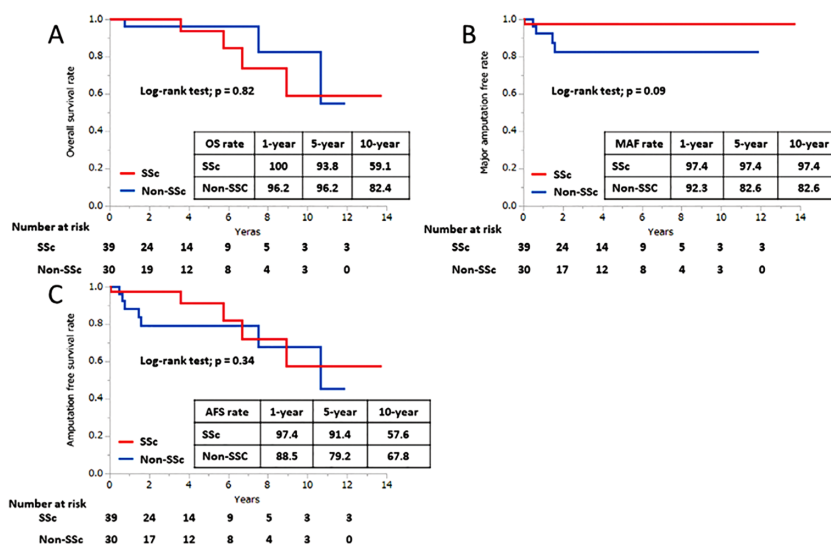
**Fig. 7** Kaplan-Meier analysis of (A) overall survival (OS), (B) major amputation-free (MAF), and (C) amputation-free survival (AFS) rates following the BM-MNC implantation in all patients with collagen diseases. Reprinted with permission from Shoji et al., Circ J 2019.

patients with no-option CLI.<sup>3)</sup> Furthermore, Miyahara et al. showed that the 5-year major AFS rate was 55.8%, whereas the 5-year AFS rate was 36.0% in patients with no-option CLI.<sup>4)</sup> Recently, overall survival and major amputation in patients with CLI tend to improve with the development of revascularization and wound care.<sup>32-36)</sup>

However, approximately 30% of patients with CLI require amputation per year, even after standard revascularization, including EVT or surgical bypass. In addition, according to the Transatlantic Intersociety Consensus II, the 1-year survival rate of patients with CLI is about 25%, indicating the very poor prognosis in patients with



**Fig. 8** Improvement in visual analog scale (VAS) scores within 6 months after the BM-MNC implantation in (A) the scleroderma (SSc) and (B) non-SSc groups. Data are expressed as medians and interquartile ranges. The P values indicate the differences before and after BM-MNC implantation. Reprinted with permission from Shoji et al., *Circ J* 2019.

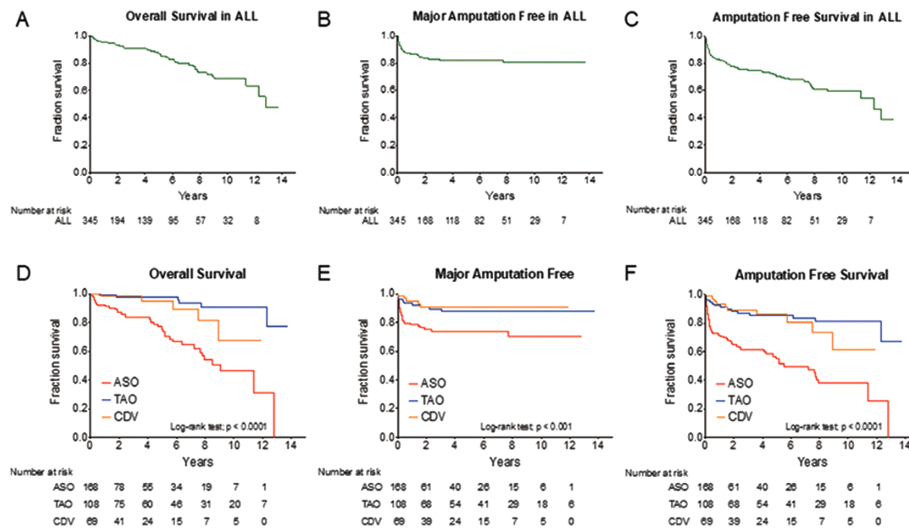


**Fig. 9** Kaplan-Meier analysis of (A) overall survival (OS), (B) major amputation-free (MAF), and (C) amputation-free survival (AFS) rates following the BM-MNC implantation in the scleroderma (SSc) and non-SSc groups. The log-rank test P values indicate differences in time-to-event between the SSc and non-SSc groups. Reprinted with permission from Shoji et al., *Circ J* 2019.

CLI.<sup>1,2)</sup> Therefore, current standard treatment alone is not sufficient, and a new therapy is required to improve the prognosis in patients with no-option CLI. Therefore, the BM-MNC implantation has been performed for patients with no-option CLI based on good results of the pilot study in 2002.

Regarding long-term prognosis reported in the J-TACT, the limb salvage rates at 1 and 5 years after the BM-MNC implantation in patients with no-option CLI caused by ASO were 79% and 74%, respectively. The AFS rates at 1 and 5 years after the BM-MNC implantation in patients with no-option CLI caused by ASO were 72% and 55%, respectively.<sup>29)</sup> So, the limb salvage rate and AFS rate in this study tended to be higher than those in a study by Miyahara et al.<sup>4)</sup> According to Matoba et al., some param-

eters, such as VAS, walking distance, and ulcer diameter, were significantly improved within 6 months after the BM-MNC implantation.<sup>28)</sup> The above results showed that the BM-MNC implantation for patients with no-option CLI caused by ASO is an acceptable and feasible therapy. However, the limb salvage rate and the improvement of clinical parameters in patients with CLI caused by ASO were less than those in patients with CLI caused by TAO or CD. Because patients with ASO are older and have more severe comorbidities and lower protein compared with patients with TAO or CD, they cannot perform sufficient rehabilitation to facilitate therapeutic angiogenesis after the BM-MNC implantation. Furthermore, we speculate that the decline of the viability and quality of BM-MNCs and exacerbation of underlying disease affect with the less



**Fig. 10** Kaplan–Meier analysis of overall survival (OS), major amputation-free (MAF), and amputation-free survival (AFS) rates following the BM-MNC implantation in all patients (A–C) and in patients with ASO, TAO, and CD (D–F). Reprinted with permission from Kondo et al., *Circ J* 2018.

efficacy of therapeutic angiogenesis in patients with ASO.

The BM-MNC implantation has been shown to have sufficient efficacy also in patients with no-option CLI caused by TAO or CD. According to the J-TACT, with regard to long-term prognosis, the limb salvage rates at 1 and 5 years after the BM-MNC implantation were 93% and 88%, respectively. Moreover, this effect persisted at 10 years after the BM-MNC implantation.<sup>31</sup> In addition, within 6 months after the BM-MNC implantation, the TAO group showed significantly high improvement in endpoints than the ASO group.<sup>28</sup> The limb salvage rates at 1 and 5 years after the BM-MNC implantation were 95% and 91%, respectively, and similar to the TAO group, the rate was maintained at 10 years after the BM-MNC implantation.<sup>28</sup>

Drug therapy and bypass surgery are recommended for patients with CLI caused by TAO or CD.<sup>1,37,38</sup> In particular, bypass surgery may sufficiently increase the limb salvage rate. In patients with TAO, the AFS rates were 91.4% at 1 year, 88.6% at 5 years, and 85.4% at 10 years after bypass surgery.<sup>5</sup> In addition, in patients with CD, the 3-year limb salvage rate was 67.2% in patients with angiitis.<sup>4</sup> However, the patency rate of bypass surgery in patients with CLI caused by TAO or CD is not sufficiently high, and some patients undergo multiple revascularization procedures owing to early reocclusion of bypass grafts. In some patients with CLI caused by TAO or CD, no peripheral arteries can be used for bypass surgery, leading to low long-term patency rates. The primary graft patency rates were 41% at 1 year, 32% at 5 years, and 30% at 10 years after the operation, and the secondary graft patency rates were 54% at 1 year, 47% at 5 years,

and 39% at 10 years in patients with TAO.<sup>5</sup> The 3-year primary and secondary patency rates of surgical bypass in patients with angiitis were 38.9% and 61.5%, respectively. In other studies, the graft patency and limb salvage rates were found to be lower in these patients than in those with CLI caused by ASO.<sup>4-6</sup> On the other hand, very few studies have shown the long-term clinical outcomes and patency rates of EVT for patients with CLI caused by TAO or CD. Many patients with CLI caused by TAO or CD cannot undergo EVT, similar to bypass surgery, owing to the poor status of peripheral arteries below the ankle. In addition, there are many patients with early recoil or reocclusion due to vascular conditions and immune mechanisms. According to previous clinical trials, the outcomes of the BM-MNC implantation are comparable to those of current revascularization strategies in patients with CLI caused by TAO or CD. We suggest that the BM-MNC implantation may be a sufficiently acceptable treatment option for patients with CLI caused by TAO or CD.

To evaluate further the efficacy of the BM-MNC implantation, a new clinical trial of advanced medical treatments was started in 2017 in Japan, initially in patients with CLI caused by TAO. This new clinical study will include additional evaluation of revascularization therapy by examining factors affecting tissue wound healing (e.g., skin perfusion pressure and transcutaneous oxygen pressure) and the angiogenic effects of therapeutic angiogenesis on ischemic tissue. In addition, in patients with CLI caused by ASO or CD, a similar study should also be performed. In particular, in patients with no-option CLI caused by ASO, we would like to evaluate the safety and efficacy of hybrid therapy with revascularization and cell



therapy. In the future, we hope that the BM-MNC implantation is practicalized as a standard treatment in clinical practice based on the results of these new clinical studies to improve the prognosis in patients with no-option CLI.

## Conclusion

Therapeutic angiogenesis using autologous BM-MNC implantation may be feasible and safe in patients with no-option CLI, particularly CLI caused by TAO and CD. Future studies should include new clinical trials for wider applications of revascularization therapy and the accumulation of additional evidence.

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## Disclosure Statement

The authors declare no competing financial interests.

## Author Contributions

Writing: KY, KS, AF, YH

Critical review and revision: all authors

Final approval of the article: all authors

Accountability for all aspects of this work: all authors

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## Appendix

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