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# A Prospective, Nonrandomized, no Placebo-Controlled, Phase I/II Clinical Trial Assessing the Safety and Efficacy of Intramuscular Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells in Patients With Severe Buerger's Disease

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Buerger's disease is a rare and severe disease affecting the blood vessels of the limbs. Adipose tissue-derived mesenchymal stem cells (ADSCs) have the potential to cure Buerger's disease when developed as a stem cell drug. In the present study, we conducted a prospective, nonrandomized, no placebo-controlled, phase I/II clinical trial with a 2-year follow-up questionnaire survey. A total of 17 patients were intramuscularly administered autologous ADSCs at a dose of 5 million cells/kg. The incidence of adverse events (AEs), adverse drug reaction (ADR), and serious adverse events (SAEs) was monitored. No ADRs and SAEs related to stem cell treatment occurred during the 6-month follow-up. In terms of efficacy, the primary endpoint was increase in total walking distance (TWD). The secondary endpoint was improvement in rest pain, increase in pain-free walking distance (PFWD), toe–brachial pressure index (TBPI), transcutaneous oxygen pressure ($TcPO_2$), and arterial brachial pressure index (ABPI). ADSCs demonstrated significant functional improvement results including increased TWD, PFWD, and rest pain reduction. No amputations were reported during the 6-month clinical trial period and in the follow-up questionnaire survey more than 2 years after the ADSC injection. In conclusion, intramuscular injection of ADSCs is very safe and is shown to prompt functional improvement in patients with severe Buerger's disease at a dosage of 300 million cells per 60 kg of body weight. However, the confirmatory therapeutic efficacy and angiogenesis need further study.

**Key words: Buerger's disease; Mesenchymal stem cells (MSCs); Adipose tissue; Autologous**

## INTRODUCTION

Buerger's disease (thromboangiitis obliterans) is an orphan disease with nonatherosclerotic, segmental inflammatory pathology that most commonly affects the small- and medium-sized arteries, veins, and nerves in the upper and lower extremities[1]. Typical patients with Buerger's disease are young men (less than 40 to 45 years) with a history of tobacco use and present with progressive claudication, ischemic ulcers, or pain at rest[1]. In severe cases, patients with Buerger's disease exhibit severe pain at rest and tissue death in the affected limbs, which eventually lead to amputation.

The most effective treatment for Buerger's disease is smoking cessation. Patient education is important, but only 43%–70% of cases manage to give up smoking[2]. Drug treatment for Buerger's disease is used to treat symptoms rather than the underlying cause and is palliative in nature. The most commonly used pharmacological agents are aspirin, cilostazol, and prostanoid. These pharmacological agents are used to improve pain-free walking distance (PFWD) through vasodilatatory and antiplatelet effects. However, the effects of these drugs are temporary and cannot prevent disease progression. To date, there is no pharmacological treatment to reverse or stop the disease progression. Therefore, new therapeutic development is essential and urgent for Buerger's disease patients. The new therapeutic agent for Buerger's disease should have 1) no adverse events (AEs), 2) an efficacy to reverse the

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disease condition through revascularization and tissue regeneration instead of temporary symptom relief, and 3) prevention or reduction of limb amputation.

Mesenchymal stem cells (MSCs) are potential new ideal therapeutic agents and hold great promise for use in tissue repair and regeneration[3,4]. Among the sources of MSCs, adipose tissue represents an abundant and practical source for obtaining autologous stem cell tissue through minimally invasive procedures. Furthermore, the safety and treatment efficacy of adipose tissue-derived stem cells (ADSCs) for various diseases have been reported in animal models and clinical trials[5,6]. We previously reported the safety and efficacy of intravenous (IV) infusion[7] and intra-articular injection[8] of autologous ADSCs. A number of studies have reported that ADSCs secrete multiple angiogenic growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor at bioactive levels[9], indicating that ADSCs can be used for therapeutic angiogenesis in a clinical setting. In this regard, the possibility of treatment for critical limb ischemia (CLI) with autologous ADSCs has been reported[10–12]. However, a clinical trial for the development of stem cell treatment using autologous ADSCs in patients with severe Buerger's disease has not yet been reported.

Accordingly, we conducted phase I and II clinical trials to investigate the safety and efficacy of autologous ADSCs at a dosage of 300 million cells per 60 kg of body weight in Buerger's disease patients who had no response to symptomatic or supportive treatment.

## MATERIALS AND METHODS

### *Study Design and Patients*

The phase I/II study of autologous ADSCs for Buerger's disease was approved by the Ministry of Food and Drug Safety with Investigational New Drug Application No. 1206 (December 18, 2007), the Institutional Review Board (IRB) at the Catholic University of Korea Seoul St. Mary's Hospital, Seoul, Republic of Korea (KCMC 08MS041), and SMG-SNU Boramae Medical Center, Seoul, Republic of Korea (20120801/06-2012-179/122). The clinical trial (ClinicalTrials.gov identifier: NCT013 02015) was started on July 31, 2008, and was completed on November 26, 2013. The subjects were given information regarding the expected efficacy and safety of the trial indicated by results from preclinical studies, that is, the Buerger's model animals significantly improved limb ischemia with human ADSCs (hADSCs) and showed no adverse effects. The subjects were informed of the study and signed a consent form. The subjects were informed that this treatment was an experimental clinical trial and that the treatment of this study was performed without any payments. The ability to pay for treatment was not a prerequisite for inclusion in the study. The phase I and II clinical trials were designed as multisite, nonrandomized, uncontrolled, and open-label studies. The inclusion criteria included subjects between 20 and 80 years of age, whose onset of Buerger's disease occurred at least 6 months prior, and who showed over 50% luminal stenosis in an angiogram. The patients classified as Rutherford class II-4, III-5, or III-6, who were experiencing rest pain or had ulcers or gangrenes, and who were not suitable subjects for revascularization or vascular bypass surgery were enrolled in the clinical trial[13]. Overall, the subjects enrolled in this clinical trial were individuals with severe cases of Buerger's disease, who were classified as Rutherford class II-4 or higher, who experienced rest pain, who had no therapeutic effect with the existing pharmacological agents, and who could be treated surgically. Individuals whose estimated life expectancy was less than 6 months due to comorbidity, or who had end-stage renal disease, or who had uncontrolled diabetes were excluded from this study. Also individuals requiring immediate amputation due to life-threatening risks and individuals with acute cardiovascular diseases, such as acute myocardial infarction and angina pectoris, were excluded.

Prior to enrollment, all subjects were checked according to the inclusion and exclusion criteria. The subjects' medical history, laboratory tests, physical examination results, and vitality signs were checked, and the efficacy endpoint examinations including the assessments of total walking distance (TWD), PFWD, and pain at rest were conducted.

### *Cell Isolation, Characterization, and Administration*

The immunophenotype, karyotyping, stability, toxicity, and tumorigenicity of cultured hADSCs were confirmed as previously described[7]. The isolation and characterization of autologous ADSCs were performed by the previously established culture protocol[7] under good manufacturing practice (GMP) conditions in the Stem Cell Research Institute of R Bio (Seoul, Republic of Korea). Adipose tissue samples were obtained through liposuction from the abdominal subcutaneous fats of the subjects a week after enrollment. Subcutaneous adipose tissues were digested with collagenase I (1 mg/ml; Gibco/Life Technologies, Grand Island, NY, USA) under gentle agitation for 60 min at 37°C and filtered through a 100-mm nylon sieve to remove cellular debris, followed by centrifugation at $470 \times g$ for 5 min. After centrifugation, the pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA)-based media containing 0.2 mM ascorbic acid and 10% fetal bovine serum (FBS; JR Scientific, Woodland, CA, USA) obtained from bovine spongiform encephalopathy-free herd. The cell fraction was cultured overnight at 37°C/5% $CO_2$ in DMEM-based media containing 0.2 mM ascorbic acid and 10% FBS. After 24 h, the cell adhesion was

checked under an inverted microscope, and nonadherent cells were removed by washing with phosphate-buffered saline (PBS). The cell medium was changed to keratinocyte serum-free medium (SFM; Invitrogen)-based media containing 0.2 mM ascorbic acid, 0.09 mM calcium, 5 ng/ml recombinant epidermal growth factor (rEGF; ProSpec, East Brunswick, NJ, USA ), and 5% FBS. The cells were maintained for 4 to 5 days until confluent (passage 0). When the cells reached 90% confluency, they were subculture expanded in keratinocyte SFM-based media containing 0.2 mM ascorbic acid, 0.09 mM calcium, 5 ng/ml rEGF, and 5% FBS until passage 3. FBS contaminants from cultured MSCs were completely removed by several washings with PBS and were verified through the test of albumin level below the measurement limit using a bovine albumin enzyme-linked immunosorbent assay (ELISA) quantitation kit (Bethyl Laboratories, Montgomery, TX, USA). Before transporting the cells for administration, aliquots of the ADSCs are tested for cell viability, fungal, bacterial, endotoxin, and mycoplasma contamination and immunophenotype for MSCs. Cell viability evaluated by trypan blue exclusion was >89%, and no evidence of bacterial, fungal, and mycoplasmal contamination was observed. The ADSCs showed a homogenous population of cells with high positive marker expression levels of CD73 and CD90 and very low negative marker expression levels of CD31, CD34, and CD45. CD73 and CD90 were expressed at a high level of >91% and >94%, respectively. Negative markers of CD31, CD34, and CD45 were expressed at a very low level of <2%. In the clinical trial, ADSCs ($1\times10^7$ cells/500 µl/syringe) were injected intramuscularly with a volume of $5\times10^6$ cells/kg (based on the patient's weight) about 3 weeks after the collection of adipose tissues. The ADSCs were injected into 30 points in the ischemic zone of the lower extremities (17 limbs from 17 patients). The subjects were instructed to visit the hospital at 1, 3, and 6 months after the injection to check various assessments for efficacy and safety.

*Safety Measures*

Safety endpoints were assessed for up to 6 months after the injection. Physical examinations, laboratory tests, and tumor marker tests were conducted on the subjects. Tolerability and AEs were also assessed. The AEs were classified based on the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 Scale (NCI CTCAE ver4.0). The incidence of adverse drug reactions (ADRs) and serious adverse events (SAEs) was also monitored.

*Efficacy Measures*

The primary efficacy endpoint, TWD, was measured using the Bruce treadmill protocol[14]. The secondary efficacy endpoint, rest pain, was measured using the visual analog scale (VAS). No rest pain was indicated as VAS 0 and the severest pain as VAS 10. The PFWD was measured using the Bruce treadmill protocol[14], with the distance measured until signs of claudication were apparent. Measurements were taken based on the toe–brachial pressure index (TBPI), transcutaneous oxygen pressure ($TcPO_2$), arterial brachial pressure index (ABPI), and angiogram according to the previous study[15]. Computed tomography (CT) angiography was used to measure the formation of collateral vessels. New collateral vessel formation was assessed as +0 (no collateral development), +1 (slight collateral development), +2 (moderate collateral development), or +3 (rich collateral development).

*Follow-Up Questionnaire Survey*

A questionnaire was sent to the subjects enrolled in the clinical trial 2 years after the injection of ADSCs as a means to examine the progression of the disease. The survey was conducted following a review by the IRB of SMG-SNU Boramae Medical Center. The principal investigator delivered the questionnaire to the subjects after obtaining informed consent via phone or mail. The assessment items in the questionnaire included 1) smoking, 2) amputation in the area where ADSCs were injected, 3) amount of medication taken for pain associated with Buerger's disease, 4) rest pain, 5) ulcer treatment in the area where ADSCs were injected, and 6) additional treatment at the administration site.

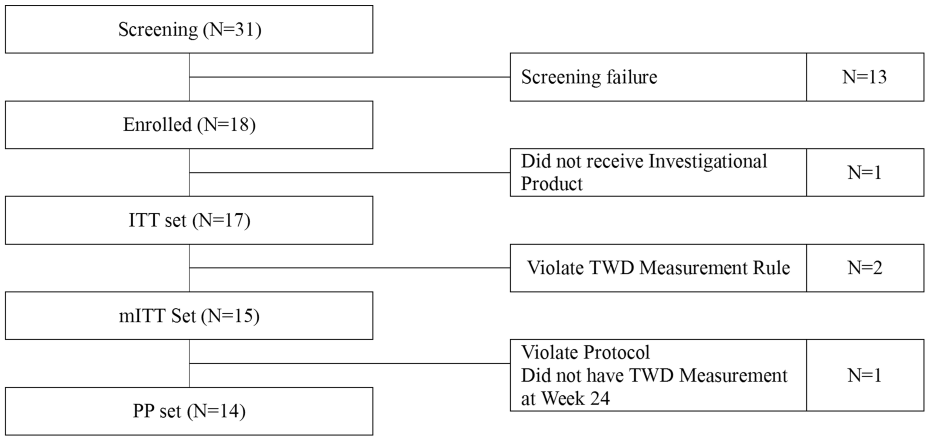
*Statistical Analysis*

A prior study of Ishida et al. was referenced to determine the number of subjects in the phase I and II clinical trials[16]. Baseline and week 4 TWD in meters from the clinical study[16] were used as a basis to calculate the current sample size. Accordingly, a sample size of 18 was obtained including 10% dropouts. Safety was assessed based on the subjects included in the intention-to-treat (ITT) analysis group, while efficacy was assessed based on the patients in the per-protocol (PP) analysis group. The missing data were substituted using the last observation carried forward (LOCF) method. The significance level for statistical analysis was set at 0.05 and considered significant when $p<0.05$. A paired *t*-test or Wilcoxon signed-rank test was run depending on the satisfaction of normality assumptions using statistical software SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Data were expressed as the mean±standard deviation (SD), median, and range (min, max).

## RESULTS

*Disposition of Patients*

A total of 31 patients were screened and gave informed consent to enter the study. Of those, 18 (58%)




| Site | Screening | Enrolled | Safety | Completed | | Discontinued | |
|---|---|---|---|---|---|---|---|
| | N | N | N | N | (%) | N | (%) |
| Total N | 31 | 18 | 17 | 17 | (94.44) | 1 | (5.56) |
| St. Mary Hospital | 14 | 8 | 8 | 8 | (100.00) | 0 | (0.00) |
| Boramae Hospital | 17 | 10 | 9 | 9 | (90.00) | 1 | (10.00) |

**Figure 1.** Patient disposition. ITT, intention to treat; mITT, modified intention to treat; PP, per protocol; TWD, total walking distance; *N*, number.

patients were eligible for the study. Of the 18 patients, 17 patients received the investigational product. One patient decided not to receive the investigational product. Therefore, 17 patients were analyzed as safety and ITT sets. Of those, two patients violated protocol for the TWD measurement rule. Therefore, 15 patients were analyzed as the modified ITT (mITT) set. Of those, one patient did not have week 24 TWD measurement. Therefore, 14 patients were analyzed as the PP set (Fig. 1).

*Demographic and Other Baseline Characteristics*

The average age of the patients was 43 years, 100% were male smokers, and 70.59% were with alcohol drinking histories (12 out of 17 patients) (see Table 1). The average age of the subjects was 42.59 ± 9.01, with individuals in their 30s and 40s accounting for more than 70% of the subjects.

*Safety*

Safety was assessed based on the 17 patients in the ITT set. Of 17 subjects, 11 (64.71%) (26 cases) experienced AEs during the clinical trial period, and treatment-emergent AEs were reported among 47.06% of the subjects (8/17 subjects, 22 cases). The details of the AEs are shown in Table 2. In the case of the 26 AEs reported, all involved pain of grade 2 or less, except for one case of grade 3 pain reported almost past the 6-month period. There were no AE-related dropouts. Moreover, the AEs were confirmed to be unassociated with the ADSC injections by the investigators who had administered the ADSCs. There were no subjects who experienced ADRs and SAEs in relation with ADSCs (Table 3). In addition, there were no statistically or clinically significant changes in the vitality signs or physical examination and laboratory test results.

**Table 1.** Demographic and Other Baseline Characteristics

| Demographic Information | ITT Set (*N* = 17) |
|---|---|
| Age (years) | |
| 20–29 | 1 (5.88%) |
| 30–39 | 7 (41.18%) |
| 40–49 | 5 (29.41%) |
| 50–59 | 3 (17.65%) |
| ≥60 | 1 (5.88%) |
| Mean ± SD | 42.59 ± 9.01 |
| Median | 44 |
| Min/Max | 24/60 |
| Gender | |
| Male | 17 (100.00%) |
| Female | 0 (0.00%) |
| Cigarette smoking | |
| Yes | 12 (70.59%) |
| No | 5 (29.41%) |
| Ceased smoking | 7 (58.33%) |
| Presently smoking | 5 (41.67%) |
| Alcohol consumption (past) | |
| Yes | 12 (70.59%) |
| No | 5 (29.41%) |
| Alcohol consumption (present) | |
| Yes | 8 (47.06%) |
| No | 9 (52.94%) |

ITT, intention to treat.

**Table 2.** Details of Adverse Events and Reactions

| No. | System Organ Class | Preferred Term | ADSCs Inj. Date | AE Start Date | AE End Date | Condition | Grade | Result | Association | Continuance of Clinical Trial | Treatment | SAE | Dropout |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 103 | Infections and infestations | Staphylococcal infection | 9/12/2008 | 9/5/2008 | 9/16/2008 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 112 | General disorders and administration site conditions | Pain | 5/27/2010 | 11/2010* | – | Continuous | Grade 3 | Continued | Clearly unassociated | Continued | No | No | Continued participation |
| 114 | Gastrointestinal disorders | Gastric ulcer | 7/28/2010 | 10/13/2010 | 10/28/2010 | Intermittent (1–2 times/week) | Grade 1 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 114 | Injury, poisoning, and procedural complications | Ligament rupture | 7/28/2010 | 11/1/2010 | – | Intermittent (ND) | Grade 1 | Continued | Clearly unassociated | Continued | Yes | No | Continued participation |
| 201 | Infections and infestations | Nasopharyngitis | 1/3/2013 | 1/26/2013 | 1/29/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 204 | Gastrointestinal disorders | Large intestine polyp | 1/24/2013 | 4/12/2013 | 4/12/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 204 | General disorders and administration site conditions | Ischemic ulcer | 1/24/2013 | 2/2013* | 8/8/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 204 | General disorders and administration site conditions | Ischemic ulcer | 1/24/2013 | 2/2013* | 8/8/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 204 | General disorders and administration site conditions | Ischemic ulcer | 1/24/2013 | 2/2013* | 8/8/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 204 | Infections and infestations | Sinusitis | 1/24/2013 | 2/2013* | 2/2013* | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 204 | Musculoskeletal and connective tissue disorders | Pain in extremity | 1/24/2013 | 3/2013* | – | Continuous | Grade 2 | Continued | Clearly unassociated | Continued | No | No | Continued participation |
| 205 | Injury, poisoning, and procedural complications | Ligament sprain | 2/1/2013 | 7/17/2013 | 7/22/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 205 | Nervous system disorders | Nerve root lesion | 2/1/2013 | 7/17/2013 | 7/22/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Gastrointestinal disorders | Diarrhea | 2/7/2013 | 2/21/2013 | 2/22/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |

(*continued*)

**Table 2.** (*Continued*)

| No. | System Organ Class | Preferred Term | ADSCs Inj. Date | AE Start Date | AE End Date | Condition | Grade | Result | Association | Continuance of Clinical Trial | Treatment | SAE | Dropout |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 208 | Gastrointestinal disorders | Dyspepsia | 2/7/2013 | 2/18/2013 | 2/19/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Infections and infestations | Nasopharyngitis | 2/7/2013 | 4/18/2013 | 4/21/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Musculoskeletal and connective tissue disorders | Back pain | 2/7/2013 | 5/31/2013 | 6/7/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Musculoskeletal and connective tissue disorders | Musculoskeletal pain | 2/7/2013 | 5/3/2013 | 5/6/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Musculoskeletal and connective tissue disorders | Musculoskeletal pain | 2/7/2013 | 5/31/2013 | 6/7/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Musculoskeletal and connective tissue disorders | Myalgia | 2/7/2013 | 3/13/2013 | 3/16/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Musculoskeletal and connective tissue disorders | Pain in extremity | 2/7/2013 | 5/3/2013 | 5/6/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 208 | Respiratory, thoracic, and mediastinal disorders | Rhinitis allergic | 2/7/2013 | 1/12/2013 | 1/19/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 211 | Nervous system disorders | Headache | 3/21/2013 | 4/2013* | 4/2013* | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 215 | Skin and subcutaneous tissue disorders | Urticaria | 5/10/2013 | 4/10/2013 | 4/12/2013 | Continuous | Grade 2 | Recovered | Clearly unassociated | Continued | Yes | No | Continued participation |
| 216 | General disorders and administration site conditions | Injection site reaction | 5/23/2013 | 5/3/2013 | 5/6/2013 | Continuous | Grade 1 | Recovered | Clearly unassociated | Continued | No | No | Continued participation |
| 217 | Musculoskeletal and connective tissue disorders | Pain in extremity | 5/16/2013 | 7/2013* | – | Continuous | Grade 2 | Continued | Unassociated | Continued | Yes | No | Continued participation |

ADSCs, adipose tissue-derived mesenchymal stem cells; AE, adverse event; SAE, serious adverse event.
*Day unknown.

**Table 3.** Summary of Adverse Events and Reactions

| | $N=17$ | |
|---|---|---|
| Adverse events and reactions | $N$ (%) | No. Cases |
| Adverse events | 11 (64.71) | 26 |
| Treatment emergent adverse events | 8 (47.06) | 22 |
| Adverse drug reactions | 0 (0.00) | 0 |
| Serious adverse events | 0 (0.00) | 0 |

*Clinical Improvement*

The primary efficacy variable used to determine the efficacy of ADSCs was the change of TWD from screening to week 24. The results are presented in Table 4 for the ITT, mITT, and PP sets. The mean changes from screening to week 24 of TWD were significantly better in every set (97.39 m, $p=0.0406$ for the ITT set; 124.21 m, $p=0.0149$ for the mITT set; and 119.91 m, $p=0.0264$ for the PP set using paired $t$-test at $\alpha=0.05$ level of significance). A similar result using the Wilcoxon signed-rank test was observed as a post hoc analysis.

The results for the secondary efficacy variable, change from screening to week 24 on the VAS, are presented in Table 5 for the ITT, mITT, and PP sets. The mean changes from screening to week 24 on the VAS were significantly better in every set (−1.76, $p=0.0289$ for the ITT set; −2.47, $p=0.0013$ for the mITT set; and −2.36, $p=0.0031$ for the PP set using paired $t$-test at $\alpha=0.05$ level of significance). A similar result using the Wilcoxon signed-rank test was observed as a post hoc analysis. The results for the secondary efficacy variable, change from screening to week 24 on the PFWD, are presented in Table 6 for the ITT, mITT, and PP sets. The mean changes from screening to week 24 on the PFWD were significantly better in every set (119.62 m, $p=0.0118$ for the ITT set; 145.57 m, $p=0.0042$ for the mITT set; and 145.14 m, $p=0.0074$ for the PP set using paired $t$-test at $\alpha=0.05$ level of significance). A similar result using the Wilcoxon signed-rank test was observed as a post hoc analysis. The results for the secondary efficacy variable, change from screening to week 24 on the ABPI are presented in Table 7 for the ITT, mITT, and PP sets. The mean changes from screening to week 24 on the ABPI for the right ankle were not significantly better in every set (0.01 mmHg, $p=0.8886$ for the ITT set; 0.01 mmHg, $p=0.8892$ for the mITT set; and −0.03 mmHg, $p=0.4080$ for the PP set using paired $t$-test at $\alpha=0.05$ level of significance). The mean changes from screening to week 24 on the ABPI for the left ankle were significantly better in every set (0.05 mmHg, $p=0.0034$ for the ITT set; 0.04 mmHg, $p=0.0105$ for the mITT set; and 0.04 mmHg, $p=0.0225$ for the PP set using paired $t$-test at $\alpha=0.05$ level of significance). A similar result using the Wilcoxon signed-rank test was observed as a post hoc analysis.

The results for the secondary efficacy variable TBPI (Table 8) and $TcPO_2$ (Table 9) were not significantly better in the ITT, mITT and PP sets using paired $t$-test and Wilcoxon signed-rank test. The results for the secondary efficacy variable, change of angiography from screening to week 24, are presented in Table 10. New collateral vessel formation was observed in only one patient in the PP set by CT angiography.

*Follow-Up Questionnaire Survey*

As for the follow-up questionnaire survey, eight out of nine subjects registered with the SMG-SNU Boramae Medical Center provided their informed consent. The longest follow-up was 2 years 8 months (Patient No. 201),

**Table 4.** Primary Efficacy Variable: Treadmill Walking Distance (TWD)

| TWD (Meters) | ITT ($N=17$) | mITT ($N=15$) | PP ($N=14$) |
|---|---|---|---|
| Visit 1 (screening) | | | |
| Mean (SD) | 300.18 (170.47) | 257.62 (117.04) | 250.06 (117.59) |
| Median | 291.20 | 270.60 | 250.00 |
| Min/Max | 35.30/764.00 | 35.30/446.00 | 35.30/446.00 |
| Visit 7 (week 24) | | | |
| Mean (SD) | 397.57 (208.04) | 381.83 (214.85) | 369.97 (217.81) |
| Median | 425.00 | 375.50 | 339.70 |
| Min/Max | 81.80/722.30 | 81.80/722.30 | 81.80/722.30 |
| Change | | | |
| Mean (SD) | 97.39 (180.25) | 124.21 (173.42) | 119.91 (179.13) |
| Median | 58.40 | 133.80 | 96.10 |
| Min/Max | −195.20/553.20 | −195.20/553.20 | −195.20/553.20 |
| 95% CI | (4.72, 190.10) | (28.17, 220.20) | (16.49, 223.30) |
| $p$ Value* | 0.0406 | 0.0149 | 0.0264 |
| $p$ Value† | 0.0569 | 0.0181 | 0.0295 |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. Change = Visit 7 (week 24) − Visit 1 (screening).
*Paired $t$-test and †Wilcoxon Signed-Rank test.

**Table 5.** Secondary Efficacy Variable: Visual Analogue Scale (VAS)

| VAS | ITT ($N=17$) | mITT ($N=15$) | PP ($N=14$) |
|---|---|---|---|
| Visit 1 (screening) | | | |
| Mean (SD) | 5.59 (2.32) | 5.87 (2.33) | 5.86 (2.41) |
| Median | 6.00 | 6.00 | 6.5 |
| Min/Max | 2.00/10.00 | 2.00/10.00 | 2.00/10.00 |
| Visit 7 (week 24) | | | |
| Mean (SD) | 3.82 (2.92) | 3.40 (2.75) | 3.50 (2.82) |
| Median | 2.00 | 2.00 | 2.00 |
| Min/Max | 1.00/10.00 | 1.00/10.00 | 1.00/10.00 |
| Change | | | |
| Mean (SD) | −1.76 (3.03) | −2.47 (2.39) | −2.36 (2.44) |
| Median | −2.00 | −2.00 | −2.00 |
| Min/Max | −7.00/5.00 | −7.00/1.00 | −7.00/1.00 |
| 95% CI | (−3.32, −0.21) | (−3.79, −1.15) | (−3.76, −0.95) |
| *p* Value* | 0.0289 | 0.0013 | 0.0031 |
| *p* Value† | 0.0309 | 0.0020 | 0.0039 |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. Change = Visit 7 (week 24) – Visit 1 (screening).
*Paired *t*-test and †Wilcoxon Signed-Rank test.

and the shortest follow-up was 2 years 3 months (Patient No. 206) after ADSC injection. Despite the long lapse of time, none of the subjects ($n=8$) had undergone amputation.

Although amputation was not included among the efficacy indicators for phase I and II clinical trials, it was possible to check for amputation in the Buerger's disease patients by assessing the affected areas during the clinical trial period. While 4 of the 17 subjects had amputations before registering for the clinical trial, none of the subjects were found to have undergone any additional amputations based on the assessments of the affected areas during the clinical trial period and the follow-up questionnaire survey conducted 2 years after the ADSC injections.

Based on data regarding the symptomatic drugs taken during the clinical trial period and prior to the follow-up questionnaire survey, changes in the amount of medication used for Buerger's disease were analyzed. As shown in Table 11, nine subjects (64.3%) stopped taking medications that they had taken prior to the clinical trial, and three subjects (21.4%) reduced the amount of medications, while two subjects (14.3%) reported no changes in the amount of medications taken.

The VAS score for pain at the end of the clinical trial was $3.5 \pm 2.9$ on average, and the average VAS score reported for a week in the questionnaire was $2.4 \pm 3.1$. This shows that there was an additional decrease of $1.1 \pm 1.9$ (32.1% decrease) in the VAS score for pain

**Table 6.** Secondary Efficacy Variable: Pain Free Walking Distance (PFWD)

| PFWD (Meters) | ITT ($N=17$) | mITT ($N=15$) | PP ($N=14$) |
|---|---|---|---|
| Visit 1 (screening) | | | |
| Mean (SD) | 217.79 (176.49) | 184.21 (114.53) | 171.40 (107.13) |
| Median | 177.30 | 143.90 | 142.80 |
| Min/Max | 35.30/762.00 | 35.30/369.50 | 35.30/369.50 |
| Visit 7 (week 24) | | | |
| Mean (SD) | 337.00 (184.69) | 329.77(179.86) | 316.54(178.92) |
| Median | 323.00 | 323.90 | 313.90 |
| Min/Max | 64.50/662.70 | 64.50/662.70 | 64.50/662.70 |
| Change | | | |
| Mean (SD) | 119.62 (173.61) | 145.57 (165.13) | 145.14 (171.35) |
| Median | 114.40 | 151.50 | 135.55 |
| Min/Max | −195.20/553.20 | −195.20/553.20 | −195.20/553.20 |
| 95% CI | (30.35, 208.90) | (54.12, 237.0) | (46.21, 244.10) |
| *p* Value* | 0.0118 | 0.0042 | 0.0074 |
| *p* Value† | 0.0079 | 0.0026 | 0.0040 |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. Change = Visit 7 (week 24) – Visit 1 (screening).
*Paired *t*-test and †Wilcoxon Signed-Rank test.

**Table 7.** Secondary Efficacy Variable: Ankle–Brachial Pressure Index (ABPI)

| ABPI (mmHg) | ITT ($N=17$) | mITT ($N=15$) | PP ($N=14$) |
|---|---|---|---|
| Visit 1 (screening) | | | |
| Right | | | |
| Mean (SD) | 0.96 (0.24) | 0.97 (0.23) | 0.98 (0.23) |
| Median | 1.07 | 1.07 | 1.08 |
| Min/Max | 0.58/1.27 | 0.58/1.27 | 0.58/1.27 |
| Left | | | |
| Mean (SD) | 0.76 (0.27) | 0.74 (0.27) | 0.75(0.28) |
| Median | 0.67 | 0.63 | 0.65 |
| Min/Max | 0.35/1.24 | 0.35/1.24 | 0.35/1.24 |
| Visit 7 (week 24) | | | |
| Right | | | |
| Mean (SD) | 0.97 (0.22) | 0.97 (0.20) | 0.95 (0.19) |
| Median | 1.00 | 1.00 | 0.96 |
| Min/Max | 0.61/1.24 | 0.63/1.23 | 0.63/1.20 |
| Left | | | |
| Mean (SD) | 0.81 (0.26) | 0.78(0.26) | 0.78(0.26) |
| Median | 0.72 | 0.70 | 0.69 |
| Min/Max | 0.46/1.28 | 0.46/1.28 | 0.46/1.28 |
| Change | | | |
| Right | | | |
| Mean (SD) | 0.01 (0.17) | 0.01 (0.18) | −0.03 (0.13) |
| Median | −0.02 | −0.03 | −0.03 |
| Min/Max | −0.24/0.50 | −0.24/0.50 | −0.24/0.17 |
| 95% CI | (−0.08, 0.09) | (−0.09, 0.11) | (−0.10 ,0.04) |
| *p* Value* | 0.8886 | 0.8892 | 0.4080 |
| *p* Value† | 0.6193 | 0.6683 | 0.3490 |
| Left | | | |
| Mean (SD) | 0.05 (0.05) | 0.04 (0.05) | 0.04 (0.05) |
| Median | 0.04 | 0.04 | 0.04 |
| Min/Max | −0.07/0.12 | −0.07/0.11 | −0.07/0.11 |
| 95% CI | (0.02, 0.07) | (0.01, 0.07) | (0.01, 0.07) |
| *p* Value* | 0.0034 | 0.0105 | 0.0225 |
| *p* Value† | 0.0040 | 0.0128 | 0.0226 |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. Change = Visit 7 (week 24) − Visit 1 (screening).
*Paired *t*-test and †Wilcoxon Signed-Rank test.

after more than 2 years of ADSC injections. However, this difference was not statistically significant ($p=0.068$). Nonetheless, two (25.0%) of the subjects participating in the survey reported zero pain (VAS score: 0) at the end of the clinical trial, and four (50.0%) of the subjects participating in the survey reported zero pain (VAS score: 0) in the questionnaire (Table 12).

As shown in Table 13, at the time of the survey, three subjects (37.5%) fully recovered from the ulcers in the area where the therapeutic agent was administered, while one subject (12.5%) responded that the ulcer was healing. However, one subject (12.5%) answered that he had developed a new ulcer. After the termination of the clinical trial, the subjects were asked whether additional treatment was administered on the areas injected with the therapeutic agent under investigation. As shown in Table 14, seven of the eight subjects (87.5%) responded that they did not undergo any additional treatments, while only one subject (12.5%) received a vascular bypass and another treatment (leech therapy).

## DISCUSSION

Buerger's disease is characterized by inflammatory occlusive vasculitis of the small- and medium-sized arteries and veins, and affects young adult smokers. Once the disease has become established, smoking cessation is the only effective way to prevent evolution of the disease and to reduce the risk of major amputations. Medical treatments with anticoagulants, vasodilators, or cilostazol may help improve the symptoms of the disease but cannot prevent disease progression[17–20]. Recognizing the need for a safe and effective alternative to discontinuation of tobacco use, phase I and II clinical trials of autologous ADSCs have been conducted for the treatment of patients with Buerger's disease who were refractory to the traditional treatment including anticoagulants, vasodilators, and surgical regime

**Table 8.** Secondary Efficacy Variable: Toe–Brachial Pressure Index (TBPI)

| TBPI (mmHg) | ITT ($N=17$) | mITT ($N=15$) | PP ($N=14$) |
|---|---|---|---|
| Visit 1 (screening) | | | |
| Right | | | |
| *n* | 8 | 8 | 8 |
| Mean (SD) | 0.38 (0.20) | 0.38 (0.20) | 0.38 (0.20) |
| Median | 0.31 | 0.31 | 0.31 |
| Min/Max | 0.12/0.70 | 0.12/0.70 | 0.12/0.70 |
| Left | | | |
| *n* | 5 | 5 | 5 |
| Mean (SD) | 0.26 (0.20) | 0.26 (0.20) | 0.26 (0.20) |
| Median | 0.23 | 0.23 | 0.23 |
| Min/Max | 0.08/0.56 | 0.08/0.56 | 0.08/0.56 |
| Visit 7 (week 24) | | | |
| Right | | | |
| *n* | 8 | 8 | 8 |
| Mean (SD) | 0.37 (0.27) | 0.37(0.27) | 0.37(0.27) |
| Median | 0.33 | 0.33 | 0.33 |
| Min/Max | 0.09/0.77 | 0.09/0.77 | 0.09/0.77 |
| Left | | | |
| *n* | 6 | 6 | 6 |
| Mean (SD) | 0.23 (0.19) | 0.23 (0.19) | 0.23 (0.19) |
| Median | 0.17 | 0.17 | 0.17 |
| Min/Max | 0.11/0.61 | 0.11/0.61 | 0.11/0.61 |
| Change | | | |
| Right | | | |
| *n* | 8 | 8 | 8 |
| Mean (SD) | −0.02 (0.20) | −0.02 (0.20) | −0.02 (0.20) |
| Median | −0.01 | −0.01 | −0.01 |
| Min/Max | −0.44/0.18 | −0.44/0.18 | −0.44/0.18 |
| 95% CI | (−0.18, 0.15) | (−0.18, 0.15) | (−0.18, 0.15) |
| *p* Value* | 0.8353 | 0.8353 | 0.8353 |
| *p* Value† | 0.9453 | 0.9453 | 0.9453 |
| Left | | | |
| *n* | 5 | 5 | 5 |
| Mean (SD) | −0.004 (0.06) | −0.004 (0.06) | −0.004 (0.06) |
| Median | 0.03 | 0.03 | 0.03 |
| Min/Max | −0.09/0.05 | −0.09/0.05 | −0.09/0.05 |
| 95% CI | (−0.08, 0.07) | (−0.08, 0.07) | (−0.08, 0.07) |
| *p* Value* | 0.8868 | 0.8868 | 0.8868 |
| *p* Value† | 1.0000 | 1.0000 | 1.0000 |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. Change = Visit 7 (week 24) − Visit 1 (screening).
*Paired *t*-test and †Wilcoxon Signed-Rank test.

and were experiencing rest pain. In other words, these patients had no therapeutic option with the current medications. Our results demonstrated that the autologous ADSC therapy was safe and prevented the risk of amputation after a 2-year follow-up. The current study also demonstrated that one-time intramuscular injection of autologous ADSCs was effective in reducing rest pain longer than 2 years in no-option Buerger's disease patients. The ADSC treatment shown in this study suggested that stem cell therapy can be used safely for Buerger's disease patients along with the use of the current medications.

Based on the conditions of Buerger's disease, a nonrandomized, noncontrolled design was selected so that all enrolled patients were treated after screening. Because it would be unethical to instruct the subjects to stop taking the medications for clinical trial purposes, in this trial the use of existing symptomatic treatment methods such as cilostazol or beraprost sodium was allowed. Safety was assessed based on the subjects included in the ITT analysis group for 24 weeks. Changes in variables after the treatment compared to screening were examined for efficacy. Although treatment assignment blinding is important to lessen the potential for bias in study results, ensuring blinding is difficult for this treatment. For these correlated data, paired sample *t*-test between screening and week 24 was an appropriate statistical method

**Table 9.** Secondary Efficacy Variable: Transcutaneous Oxygen Pressure ($TcPO_2$)

| $TcPO_2$ (mmHg) | ITT ($N=17$) | mITT ($N=15$) | PP ($N=14$) |
|---|---|---|---|
| Visit 1 (screening) | | | |
| Mean (SD) | 51.72 (25.34) | 55.82 (23.61) | 57.71 (23.30) |
| Median | 44.50 | 52.80 | 54.85 |
| Min/Max | 8.20/104.30 | 28.00/104.30 | 28.00/104.30 |
| Visit 7 (week 24) | | | |
| Mean (SD) | 47.51 (14.57) | 48.60 (13.03) | 49.71 (12.76) |
| Median | 51.30 | 51.30 | 51.65 |
| Min/Max | 18.50/65.00 | 20.5/65.00 | 20.50/65.00 |
| Change | | | |
| Mean (SD) | −4.21 (25.35) | −7.22 (25.35) | −7.99 (26.13) |
| Median | −3.00 | −5.50 | −7.05 |
| Min/Max | −52.30/36.60 | −52.30/36.60 | −52.30/36.60 |
| 95% CI | (−17.25, 8.82) | (−21.26, 6.82) | (−23.08, 7.09) |
| *p* Value* | 0.5032 | 0.2887 | 0.2730 |
| *p* Value† | 0.5791 | 0.2769 | 0.2412 |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. Change = Visit 7 (week 24) – Visit 1 (screening).
*Paired *t*-test and †Wilcoxon Signed-Rank test.

for the analyses of primary and continuous secondary efficacy variables.

In terms of safety of autologous ADSCs, no SAEs were observed following the intramuscular injections of ADSCs in Buerger's disease patients. Also no ADRs occurred, and none of the subjects dropped out from the study during the clinical trial period. These results confirm and expand the safety of ADSCs, which was previously confirmed in patients with spinal cord injury[7] or osteoarthritis[8]. In line with our results, the administration of stem cells has been reported to be safe in numerous studies, confirming again the safety of stem cell drug[21–25].

The efficacy results of this study demonstrated the clinical benefit of autologous ADSCs in the treatment of patients with Buerger's disease. Treatment with ADSCs was superior on week 24 compared to screening in

**Table 10.** Secondary Efficacy Variable: Angiography

| Visit 7 (Week 24) | +0 [*n* (%)] | +1 [*n* (%)] | +2 [*n* (%)] | +3 [*n* (%)] |
|---|---|---|---|---|
| ITT set ($N=15$*) | | | | |
| Visit 1 (screening) | | | | |
| +0 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| +1 | 0 (0.00) | 3 (20.00) | 0 (0.00) | 0 (0.00) |
| +2 | 0 (0.00) | 0 (0.00) | 5 (33.33) | 1 (6.67) |
| +3 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 6 (40.00) |
| *p* Value | 0.8013 | | | |
| mITT set ($N=15$) | | | | |
| Visit 1 (screening) | | | | |
| +0 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| +1 | 0 (0.00) | 3 (20.00) | 0 (0.00) | 0 (0.00) |
| +2 | 0 (0.00) | 0 (0.00) | 5 (33.33) | 1 (6.67) |
| +3 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 6 (40.00) |
| *p* Value | 0.8013 | | | |
| PP set ($N=14$) | | | | |
| Visit 1 (screening) | | | | |
| +0 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| +1 | 0 (0.00) | 2 (14.29) | 0 (0.00) | 0 (0.00) |
| +2 | 0 (0.00) | 0 (0.00) | 5 (35.71) | 1 (7.14) |
| +3 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 6 (42.86) |
| *p* Value | 0.8013 | | | |

ITT, intention to treat; mITT, modified intention to treat; PP, per protocol. +0: no collateral development; +1: slight collateral development; +2: moderate collateral development; +3: rich collateral development; McNemar Bowker's test.
*Two patients not included.

**Table 11.** Changes in the Intake of Pain Medications by the Clinical Trial Subjects

| Patient No. | Before ADSCs Administration (The Intake of Medications at Day of Screening) | 6 Months After ADSCs Administration | 2 Years After ADSCs Administration | Remarks |
|---|---|---|---|---|
| 103 | Oxycodone hydrochloride<br>Sarpogrelate hydrochloride<br>Beraprost sodium<br>Tramadol hydrochloride<br>Aspirin (500 mg) | Oxycodone hydrochloride<br>Cilostazol<br><br><br>Aspirin (100 mg) | Unchecked | Reduced dose |
| 106 | Cilostazol | Cilostazol | Unchecked | No change |
| 107 | Sarpogrelate hydrochloride<br>Limaprost alfadex | | Unchecked | Ceased intake |
| 109 | Cilostazol | Cilostazol | Unchecked | No change |
| 110 | Beraprost sodium<br>Limaprost alfadex<br>Aspirin | <br><br>Aspirin | Unchecked | Reduced dose |
| 201 | None* | Acetaminophen (325 mg)<br>Tramadol hydrochloride (37.5 mg)<br>(Temporary intake before Visit 7) | Acetaminophen (162.5 mg)<br>Tramadol hydrochloride (18.75 mg) | Reduced dose |
| 202 | None† | Beraprost sodium<br>Aspirin | None | Ceased intake |
| 204 | Cilostazol | Cilostazol | None | Ceased intake |
| 205 | Beraprost sodium<br>Aspirin | Beraprost sodium<br>Aspirin | None | Ceased intake |
| 208 | Beraprost sodium | Beraprost sodium | None | Ceased intake |
| 211 | None‡ | None | None | Ceased intake |
| 215 | Beraprost sodium | None | None | Ceased intake |
| 216 | Beraprost sodium | None | None | Ceased intake |
| 217 | Beraprost sodium<br>Kallidinogenase | Beraprost sodium<br>Kallidinogenase | None | Ceased intake |

ADSCs, adipose tissue-derived mesenchymal stem cells.
*The patient took medications before the day of screening including Prena Tab (methylprednisolone), Retonase Tab (streptokinase–streptodornase), and Ibuprofen Tab 400 mg.
†The patient took medications before the day of screening including Celebrex Cap 200 mg (celecoxib), Aspirin Protect Tab 100 mg (aspirin), Mevalotin Tab, and Berasil Tab (beraprost sodium).
‡The patient took Coumadin 5 mg before the day of screening.

demonstrating a significantly better improvement on TWD and PFWD. In the phase I and II clinical trials for ADSCs, the walking distance was measured according to the Bruce treadmill protocol. The Bruce treadmill protocol involves increasing the speed and incline over time, that is, a gradual increase in the exercise load over time, and the walking distance inevitably decreases over time. In the case of clinical trials using cilostazol, the constant walking speed and gradual increase of incline were used, while in this study the speed and incline were increased gradually for measuring the maximal walking distance (MWD) (=TWD) and PFWD[26–28]. An increase in MWD by 95.7 m, from 236.9 to 332.6 m (40.40% increase), and an increase in PFWD by 95.5 m, from 211.4 to 306.9 m (45.18% increase), were reported[26]. An increase of 109 m, from 241 to 350 m (45.23%), for MWD and an increase of 94 m, from 124 to 218 m (75.81%), for PFWD were observed[27]. In addition, an increase of 73 m, from 262 to 335 m (27.86%), for MWD was reported[28]. In this study, the ADSCs resulted in 48% and 85% increases in MWD and PFWD on condition of gradually increasing walking speed and incline. Although direct comparison is not possible because of different test conditions of MWD and PFWD used in this and other studies[26–28], the results of TWD and PFWD indicated that ADSCs can improve the walking distance in severe Buerger's disease patients. In addition, since Buerger's disease patients in this study had pains and walking disturbance despite taking medications such as cilostazol and beraprost sodium before

**Table 12.** Results of the Follow-up Questionnaire Items on Rest Pain Measured by the Visual Analog Scale ($N=8$)

| Category | Mean ± SD | Median | Min/Max |
|---|---|---|---|
| End of clinical trial | 3.5 ± 2.9 | 4 | 0/7 |
| In the past week | 2.4 ± 3.1 | 0.5 | 0/7 |
| Change | −1.1 ± 1.9 | 0 | −5/0 |
| *p* Value | | 0.068 | |

**Table 13.** Results of the Follow-Up Questionnaire Items on Ulcer Treatment

| Questionnaire Results | $n=8$ | Changes in Ulcer After Clinical Trial | $n=8$ |
|---|---|---|---|
| No ulcers | 3 (37.5%) | No existing ulcers | 4 (50.0%) |
| Newly occurring ulcers | 1 (12.5%) | Existing ulcers | 4 (50.0%) |
| Amid recovery | 1 (12.5%) | Amid recovery | 1 (25.0%) |
| Full recovery from ulcers | 3 (37.5%) | Full recovery from ulcers | 3 (75.0%) |
| Total | 8 (100.0%) | Total | 8 (100.0%) |

ADSC injection, we believe that the differences of TWD and PFWD between the screening day and week 24 are purely the effect of stem cell treatment.

The other efficacy indicators in this clinical trial were rest pain assessed based on the VAS as well as the ABPI, TBPI, and $TcPO_2$. Reduced rest pain resulting from ADSCs was observed during the clinical trial period, and further reduction in rest pain was reported in the follow-up questionnaire survey conducted 2 years later. To be more specific, 40.5% reduction in rest pain was observed during the clinical trial period, and an additional 33% reduction was reported 2 years afterward. These results clearly demonstrated that ADSCs indeed relieved the rest pain, which could not be ameliorated by the existing drugs, and thereby stopped or reduced the amount of drugs taken. Treatment with ADSCs was superior on week 24 compared to screening in demonstrating a significantly better improvement on the left ABPI. However, statistically significant results were not obtained in regard to TBPI. Since many patients did not have TBPI measurement at screening and week 24, it was difficult to make any statistical and clinical robust assessment for this efficacy variable. Taken together, the results of the VAS and left ABPI provided further support for the results of the primary efficacy variable.

Buerger's disease is a progressive, irreversible disease accompanied by gradually aggravated symptoms that eventually results in amputation of the affected area regardless of treatment. In a previous study, major amputations were performed on 5 limbs (14.3%) of the 35 lower limbs among the 31 patients in the group that received artery bypass surgery[29]. A previous study reported that 14% of the 100 patients with Buerger's disease received sympathectomy and amputation surgery simultaneously, and an additional 18% of the subjects received amputation surgery just a few months later due to recurrence of symptoms[30]. Additionally, in a clinical trial of iloprost conducted with Buerger's disease patients, amputation surgery was performed on 6% of the subjects despite administration of iloprost[31]. The patients enrolled in this trial had high risk of amputation because of nonresponse to existing drugs and other therapeutic options. Therefore, evaluating the amputation rate is an important parameter for clinical benefits of ADSCs. In the current study, no amputations were reported during the 6-month clinical trial period and in the follow-up questionnaire survey more than 2 years after the ADSC injection. This result indicates that ADSCs present a better possible treatment option in preventing amputation for patients with severe Buerger's disease.

The mechanism of the therapeutic effects of ADSCs is completely different from that of the conventional agents used for symptomatic treatment. The mechanism of actions of conventional agents such as cilostazol and argatroban involves vasodilation, thrombosis prevention, and platelet aggregation inhibition, with the purpose of alleviating or inhibiting disease symptoms resulting from arterial obstruction by preventing thrombosis or dilating blood vessels[32–34]. On the other hand, ADSCs are the type of therapeutic agent that works locally for angiogenesis and strengthening of blood vessels, which is also a known mechanism of other stem cells[35,36]. The mechanism of ADSCs used in this study was confirmed in our previous study through in vitro and in vivo animal studies[7]. In the previous study, we reported that ADSCs were differentiated into endothelial cells and prevented leg amputations by increasing the density of capillaries and the blood flow in ischemic limb model animals. The therapeutic angiogenic effect of ADSCs was through the secretion of diverse angiogenic factors including VEGF (data not shown). In line with our results, many studies have revealed the antigenic and therapeutic effect of VEGF through preclinical and clinical studies[37–43]. A study reported that an injection of recombinant VEGF protein

**Table 14.** Results of the Follow-Up Questionnaire Items on Additional Treatment on the Administration Site

| Category | $n=8$ |
|---|---|
| Did not receive any other treatments | 7 (87.5%) |
| Sympathectomy | 0 (0.0%) |
| Vascular bypass | 1 (12.5%) |
| Amputation | 0 (0.0%) |
| Other treatments | 1 (12.5%) |
| Total | 8 (100.0%) |

(rVEGF) in laboratory animals resulted in the promotion of angiogenesis and that angiogenesis occurred at a higher rate when rVEGF was injected in combination with angiopoietin-2 (Ang-2)[37]. Furthermore, a number of studies reported that the injection of naked VEGF DNA effectively treated patients with peripheral arterial disease and Buerger's disease in terms of improvement in clinical symptoms and ABI, generation of new blood vessels, and enhancement of the walking function[38–43]. In this study, we investigated the correlation in the results of therapeutic angiogenesis between our previous preclinical study and clinical study. Unfortunately, there were little to no direct angiogenesis effects observed in this clinical trial in contrast with the preclinical study. In the clinical trial, angiogenesis was observed in only one subject based on an angiogram, while the diverse symptoms of Buerger's disease were ameliorated, and no amputation was observed. In line with our results, a study reported that amputation was prevented for all 37 subjects, yet angiogenesis was observed in only 23 subjects based on an angiogram after 24 months of administrating bone marrow-derived mesenchymal stem cells (BM-MSCs) and nucleated cells to patients with CLI[44]. In another study, angiogenesis was observed by angiography in only 3 patients (14%) out of 21 patients saved from amputation after 6 months of administrating the nucleated cells of the bone marrow to CLI patients[45]. Although functional improvements were shown in several parameters, the reasons for not observing angiogenesis in this trial in comparison with the previous study[11] can be due to the technological limitations in assessing the formation of microvessels. Digital subtraction angiography (DSA) was used in the previous study[11], and CT angiography was used in this study. The sensitivity of CT angiography is lower than that of DSA. In addition, even DSA cannot detect a microvessel less than 0.2 mm[45]. Therefore, the reasons for not observing angiogenesis in the current study can be due to the sensitivity and technical limitation of CT angiography. In addition, the short observation period (6 months) can be another reason. Further research is needed to explore the reasons for not observing angiogenesis.

## CONCLUSION

Collectively, this study was conducted to investigate the safety and efficacy of intramuscular injection of autologous ADSCs in patients with severe Buerger's disease. The results of the phase I and II clinical trials demonstrated that ADSCs are highly safe for administration in Buerger's disease patients as no ADRs occurred, and none of the subjects dropped out from the study during the clinical trial period. In terms of efficacy, administration of ADSCs improves walking capacity, reduces rest pain, and potentially prevents the risk of amputation. However, further research is necessary for confirmation of the mechanism and efficacy of ADSCs.

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