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HUMAN CLINICAL ARTICLE

STEM CELLS TRANSLATIONAL MEDICINE

# Phase IV postmarketing surveillance study shows continued efficacy and safety of Stempeucel in patients with critical limb ischemia due to Buerger's disease

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## Abstract

Buerger's disease or thromboangiitis obliterans is a type of obstructive vascular diseases categorized as vasculitis and usually present in 95% of young smoker men. The main pathogenetic mechanism is interplay between immune system and inflammation. Earlier our phase II study has shown that Stempeucel is safe when injected at 2 million cells/kg body weight by virtue of its anti-inflammatory, immunomodulatory, and angiogenetic properties. The present study was conducted to further assess the safety and efficacy of Stempeucel in critical limb ischemia due to Buerger's disease after obtaining approval from Indian FDA based on the data generated in the phase II study. This is an open label, multicenteric phase IV PMS study conducted across India with experienced vascular surgeons. Fifty patients of critical limb ischemia due to Buerger's disease with Rutherford III-5 or III-6 were included in the study and each individual received a dose of 2 million cells/kg body weight of Stempeucel in the calf muscles and around the ulcer. These patients were evaluated over 12 months from drug administration. The present study showed the continued long term efficacy over a period of 12 months follow up in these patients corroborating the result obtained in the previous phase II studies. There was significant improvement in rest pain, ankle systolic pressure, and ankle brachial pressure index with accelerated ulcer healing. In conclusion, the present study shows that the intramuscular administration of Stempeucel continues to be safe, tolerable, and effective alternative treatment in patients with Buerger's disease.

National Institutes of Health and Clinical Trials Registry–India (CTRI) Web site: CTRI/2018/02/011839.

**KEYWORDS**
angiogenesis, ankle brachial pressure index, critical limb ischemia, mesenchymal stromal cells, rest pain score, Rutherford III-5 or III-6

### Lessons learned

- Stempeucel showed efficacy and safety after a single administration of cells at the site of ischemia in "no-option" patients with critical limb ischemia due to Buerger's disease.
- Stempeucel is capable of alleviating a patient's symptoms after standard treatment has failed.
- Clinical benefits include reduction of rest pain, increased healing rates of nonhealing ulcers, and decreased need for amputation.
- Stempeucel is an effective alternative to achieve rapid therapeutic angiogenesis.

### Significance statement

Stempeucel consists of pooled, allogeneic, mesenchymal stromal cells derived from bone marrow of healthy adult volunteers. The idea behind using allogeneic mesenchymal stem cells is to initiate a regressive process upon the inflammation in critical limb ischemia due to Buerger's disease and then to mount an angiogenic and anti-inflammatory process to improve effectiveness in no-option patients.

## 1 | INTRODUCTION

Buerger's disease, also known as thromboangiitis obliterans (TAO), is a serious medical condition characterized by segmental nonatherosclerotic inflammatory disorder that primarily involves the small and medium sized arteries, veins, and nerves of the extremities.[1] The disease typically presents in patients around 45 years of age and is more frequent in male smokers but also seen in women.[2] Most of the patients are heavy smokers, suggesting that tobacco smoking is the most common risk factor, but Buerger's disease may also develop as a result of chewing tobacco or use of marijuana.[3,4] Tobacco use is strongly connected to the onset, progression, and prognosis of this disease.[1] An additional risk factor may be having severe periodontal disease and chronic anaerobic periodontal infection, which is seen in nearly two thirds of patients with TAO.[5] The disease progression in critical limb ischemia (CLI) is characterized by rest pain, tissue loss, and gangrene, which may lead to major or minor amputation. Amputation may be required when the possibility of revascularization surgery is small in these patients because of diffuse segmental involvement and the distal nature of the disease.[6] The disease is observed worldwide with approximately 0.4 million people in India.[7,8]

Several clinical trials in the past decade have demonstrated the safety and efficacy of injection of bone marrow mononuclear cells (BMMNCs) or bone marrow-derived mesenchymal stem cells (BMMSCs) in inducing angiogenesis and improving the functional activity of ischemic limbs and limb salvage.[9,10] BMMSCs are known to secrete a number of angiogenic factors and have shown to form capillary-like structures in an in vitro Matrigel assay.[11] The probable mechanism of action is the anti-inflammatory and angiogenic response of BMMSCs through their paracrine activity when injected locally in the calf muscles and around the ulcer in the affected limb. The most common angiogenic factors implicated are vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), stromal-derived factor-1, interleukin (IL)-8, IL-6, hepatocyte growth factor (HGF), and transforming growth factor beta-1 (TGF-β1), which plays a role in neoangiogenesis.[12]

Our previous[8] phase II study showed that use of Stempeucel was safe and efficacious at a dose of 2 million cells per kilogram body weight in patients with CLI due to Buerger's disease. Given the limitation of the current therapies, high rate of amputations, gravity of the disease, and major unmet medical need in the country, along with the safety and efficacy data generated in the phase II study, the Indian Food and Drug Administration (FDA) (Central Drugs Standard Control Organization) granted approval for the conduct of a phase IV study.

This report highlights clinical data from 1 year of follow-up of the 50 patients who completed the phase IV study.

## 2 | MATERIALS AND METHODS

### 2.1 | Preparation of Stempeucel

The investigational medicinal product was BMMSCs, which were obtained from bone marrow aspirates from healthy donors who were not human leukocyte antigen (HLA) matched to the recipients. The volunteers for bone marrow donation were tested according to Indian Council of Medical Research (ICMR) guidance for healthy bone marrow donor screening. Mesenchymal stromal cells (MSCs) were isolated from the donor's BMMNCs using the density gradient separation method and cultured. The cells were expanded *in vitro* to manufacture the required number of cells. We have a two-tier cell banking system

consisting of a donor master cell bank (MCB) and a working cell bank (WCB). BMMSCs were isolated by plastic adherence from the donor's BMMNCs and cultured until passage 1. A donor MCB constituted from MSCs from an individual bone marrow sample was created and maintained under cryopreserved conditions. Subsequently, a WCB was prepared by combining an equal number of MSCs from three donors pooled together, cultured and expanded until passage 3, and cryopreserved. The pooled WCB was further expanded for additional passages 5 for manufacturing Stempeucel (U.S. patent number 8956862, dated February 17, 2015). Stempeucel is manufactured depending on requirement, and a total of seven Stempeucel batches were manufactured in the Good Manufacturing Practices facility for the study. Cryopreserved Stempeucel units were taken out and assessed for identity, purity, impurity, strength/potency, sterility, safety, and genetic stability against stringent in-house specifications before being released for the study. The potency of Stempeucel, which has a direct role in clinical significance, was evaluated via critical validated test parameters—viable cell count, viability, and VEGF potency assay.

## 2.2 | Viable cell count

Cryopreserved Stempeucel in a 50-mL cryobag (Miltenyi Biotec Gmbh, Bergisch Gladbach, Germany) was thawed at 37°C in a water bath and revived by adding 35 mL PlasmaLyte-A (Baxter, Deerfield, IL). The revived content was transferred to a 50-mL centrifuge tube. The contents were mixed gently, and an aliquot of 0.5 mL was sampled for quantification of viable cell count using a Vi-cell XR automated cell viability analyzer (Beckmann Coulter, Brea, CA) by the trypan blue dye exclusion method. Viable cell count was performed using validated cell type settings specific for the Stempeucel. Sample placed in the sample carousel (0.5 mL) was aspirated, mixed, and stained with equal volume of trypan blue dye (Beckmann Coulter). The stained sample was loaded, and images were captured using the video-based technology. An average of data from 50 images was reported by the equipment as total viable cells per milliliter of the sample.

The nonviable cell appeared dark as the trypan blue entered the permeable cell membrane, whereas viable cells appeared bright since no trypan blue entered the intact cell membrane. The viable and nonviable cells were distinguished by the equipment based on the two critical cell type settings: viable cell spot area and viable cell spot brightness.

## 2.3 | Viability assay

A viability assay was performed by staining with 7-aminoactinomycin D (7-AAD) fluorescent dye (BD Biosciences, San Jose, CA) and analyzed using a flow cytometer. Sample (0.1 mL) was aliquoted from the revived Stempeucel in 50-mL centrifuged tube and diluted with phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Waltham, MA) to adjust the cell concentration between 400 to 500 cells per microliter. Sample (200 μL) containing approximately $10^5$ cells was loaded into two wells of U-bottom 96-well plates. 7-AAD fluorescent dye (2 μL) was added to one of the well with sample and incubated for 10 minutes at room temperature in dark. Another well containing the sample without the 7-AAD fluorescent dye was used as unstained/negative control. Both the stained and unstained samples were acquired in the red fluorescence channel on the Guava EasyCyte plus flow cytometer (Luminex, Austin, TX) using CytoSoft 2.0 software. The acquisition and analysis were performed in a dot plot with red fluorescence in the *x*-axis and side scatter in the *y*-axis.

7-AAD dye enters the permeable cell membrane of nonviable cells, binds only to double-stranded DNA by intercalating between G-C base pairs, and produces fluorescence signals detected in the red fluorescence channel. Viable cells with intact cell membrane are impermeable by the 7-AAD dye and produce no fluorescence signal in the red fluorescence channel.

## 2.4 | VEGF assay

Revived Stempeucel in the 50-mL centrifuge tube was centrifuged at 1200 *g* for 10 minutes. The cell pellet obtained was suspended in PBS, and a cell count was performed. From the cell suspension, 1 million viable cells were added to 10 mL sterile complete medium containing 85% KnockOut Dulbecco's modified Eagle's medium (DMEM-KO) (Thermo Fisher Scientific), 10% fetal bovine serum (GE Healthcare, Auckland, New Zealand) 1% glutamine (Thermo Fisher Scientific), and basic fibroblast growth factor (FGF) (Sigma-Aldrich, St. Louis, MO) at 2 ng/mL concentration and seeded in a T75 flask. The seeded flask was incubated in a humidified incubator at 37°C with 5% $CO_2$. After 72 hours, spent medium was collected, cultures were harvested, and a cell count was performed. Collected spent medium was centrifuged at 1500 *g* for 5 minutes to remove the cell debris, and the supernatant was filtered using 0.22-μm sterile syringe filter and stored at −80°C as aliquots of 1 mL. Stored spent medium aliquot was thawed, diluted 1:6 with DMEM-KO, and subjected for VEGF enzyme-linked immunosorbent assay (ELISA) using the ELISA kit (R&D Systems, Minneapolis, MN). The VEGF value obtained was reported as nanograms per milliliter per million cells.

## 2.5 | Study design

This prospective study was designed as an open-label, multicenter, postmarketing surveillance (PMS) study to assess the safety and efficacy of intramuscular administration of Stempeucel in patients with CLI due to Buerger's disease. The protocol was approved by the Indian FDA and institutional ethics committees of all participating sites. The study was conducted as per the International Council for Harmonization Good Clinical Practice guidelines, principles of the Declaration of Helsinki, Schedule Y of the 1945 Drugs and Cosmetic Act, and Ethical Guidelines for Biomedical Research on Human Participants, ICMR 2006.

After taking informed consent, a total of 50 patients of lower extremity CLI due to Buerger's disease were enrolled into the study. However, a total of 200 patients were planned to be

enrolled in the study, and an interim analysis of the data has been done after enrollment of 50 patients in the study. All patients were injected with Stempeucel at a dose of 2 million cells per kilogram body weight in the calf muscles and around the ulcer. The patients were followed up for a duration of 12 months from the time of administration of Stempeucel for both safety and efficacy. Thereafter, the patients will be followed up for another 3 years for safety only. The visits scheduled for the study were the baseline visit (visit 1) when Stempeucel was administered, followed by 1 month (visit 2), 6 months (visit 3), and 12 months (visit 4) after administration of Stempeucel. The study was sponsored by Stempeutics Research. The study was registered on the National Institutes of Health and Clinical Trials Registry-India (CTRI) Web site (CTRI number: CTRI/2018/02/011839).

## 2.6 | Selection of patients

Patients of either sex of aged at least 18 years with established clinical diagnosis of CLI due to Buerger's disease as per Rutherford classification (Rutherford III-5 or III-6) with rest pain and/or ulcers in the affected limb and who were not eligible for or had failed traditional revascularization treatment as qualified by the physician were included in the trial. Patients with known hypersensitivity to the excipients of the Stempeucel—dimethyl sulfoxide (DMSO) or human serum albumin (HSA)—were excluded from the trial.

## 2.7 | Preparation and reconstitution of Stempeucel for administration

Cryopreserved Stempeucel (200 million cells) was stored in 15 mL of PlasmaLyte-A (multiple electrolytes injection, type 1, U.S. Pharmacopeia) containing 5% HSA (Baxter) and 10% DMSO (Sigma-Aldrich) in a Cryocyte bag (MacoPharma, Mouvaux, France) at −185°C to −196°C. The cryopreserved cells were thawed in a sterile distilled water bath at 37°C. The cryobag was gently rocked to mix the suspension for 3-4 minutes until it thawed. PlasmaLyte-A (35 mL) was added aseptically into the cryobag to make up the volume to 50 mL. Thus, the cryobag in the final suspension contained 200 million cells per 50 mL or 2 million cells per 0.5 mL Stempeucel along with 1.6% HSA with 3.33% DMSO in multiple electrolytes (PlasmaLyte-A) solution. Stempeucel reconstitution was done by a trained person. Administration of Stempeucel was done through disposable sterile syringes, and it was used within 60 minutes after reconstitution.

## 2.8 | Intramuscular injection protocol

At least 60 minutes before administrating Stempeucel, patients were injected intravenously with premedication (100 mg injection of hydrocortisone and 45.5 mg injection of pheniramine maleate). Stempeucel was administered intramuscularly as 40-60 multiple injections in the gastrocnemius muscle of the ischemic lower limb (40-60 sites, distributed in an area of 10 × 6 cm, 1-1.5 cm deep, and 0.5-1.0 mL of Stempeucel per site) (supplemental online Figure 1). Injection points were separated by 1 cm from each other. In case of ulcers, 2 mL (out of the total calculated volume for the patient) was administered around the ulcers as multiple intramuscular injections (4-6 sites; 0.3-0.5 mL of Stempeucel per site). On discharge, the patients were given standard protocol of care for the disease as per the investigator's discretion.

## 2.9 | Study endpoints

### 2.9.1 | Efficacy evaluation

Endpoint for the efficacy included evaluation of rest pain score using visual analog scale (on a scale of 0-10, where 0 was "no pain" and 10 was "severe pain"), ankle systolic pressure (ASP), ankle brachial pressure index (ABPI) measured using Doppler, and ulcer status (assessed as healed, improved, or not healed), which was photographically documented to avoid bias at 1, 6, and 12 months.

### 2.9.2 | Safety evaluation

Safety assessment included evaluation of adverse events (AEs), treatment-emergent AEs (TEAEs), occurrence of clinical abnormality at site of injection, calf area, vital signs, physical examination, and laboratory investigations at 1, 6, and 12 months.

## 2.10 | Data collection

Data collection was performed using electronic case record form. Third-party monitors verified the data with the source notes independent of investigators.

## 2.11 | Statistical analysis

### 2.11.1 | Sample size calculation

The Indian FDA provided approval for conduct of a phase IV study of Stempeucel with a condition of submitting efficacy and safety data of 200 patients. An interim analysis was performed at 12 months of follow-up after enrollment of 50 patients.

### 2.11.2 | Analysis of data

SAS package (version 9.2; SAS Institute Inc., Cary, NC) was used for statistical analysis. Data are presented as means ± SD. TEAEs are

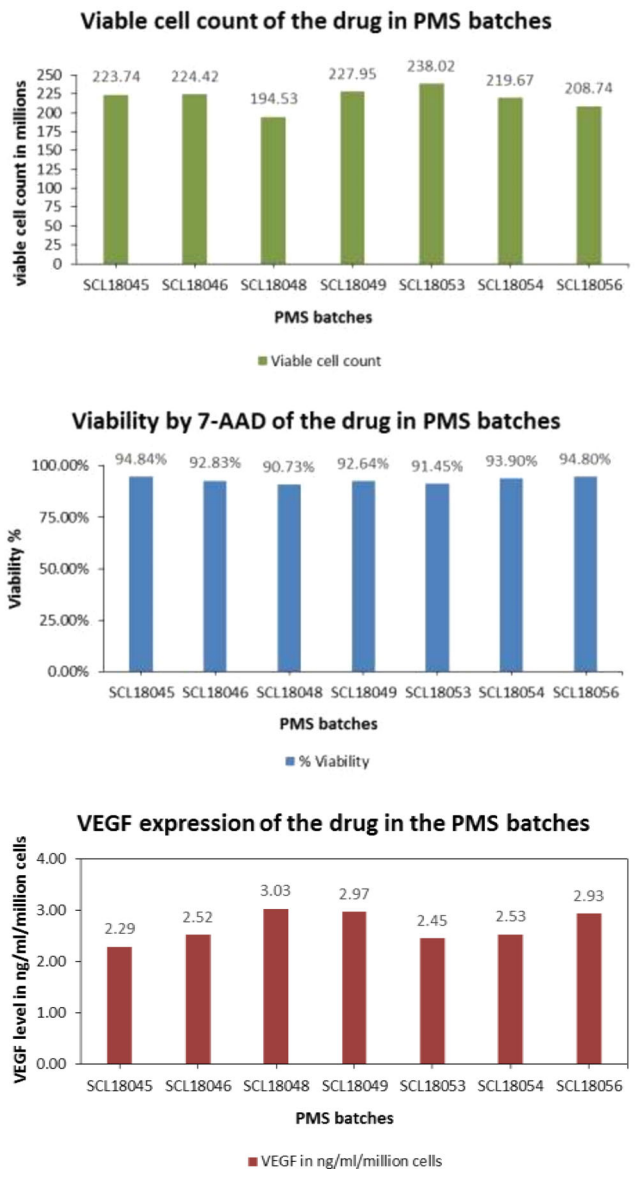


**FIGURE 1** Potency data of Stempeucel in PMS batches. Abbreviations: 7-AAD, 7-aminoactinomycin D; PMS, postmarketing surveillance; VEGF, vascular endothelial growth factor

summarized descriptively by total number of AEs in each group by system organ class (SOC). Normality of continuous data was tested using the Shapiro-Wilk test. Change in rest pain, ankle pressure, and ABPI from baseline to each visit were evaluated using Wilcoxon signed-rank test or paired *t* test based on normality of the data. Fisher's exact test was used to assess statistically significant difference in the ulcer healing status from baseline to each follow-up visit. The efficacy parameters were also analyzed by using a generalized estimating equation (GEE) model with longitudinal analysis[8,13,14] and chi-square test as appropriate. A value of $P < .05$ was considered statistically significant. Efficacy data are presented for modified intention to treat population, which represents the patients who had at least one postbaseline efficacy data point.

## 3 | RESULTS

### 3.1 | Potency of the drug product

All seven Stempeucel batches were released for the PMS study after meeting the established in-house specifications. We choose three important parameters—the viable cell count, cell viability percentage, and quantity of VEGF secretion—as the potency of Stempeucel, which has a direct role in clinical significance. The viable cell yield varied between 195 and 238 million cells, much above the minimum limit (Figure 1). Furthermore, all the batches have shown good viability above 90% with maximum viability of 95% (Figure 1). For viability, the Vi-cell XR cell counter captured 50 images for each sample, and both individual image data and averages of the 50 images were provided by the equipment and were taken as a viability count of the sample. Supplemental online Figure 2 shows representative images of the data captured. The secretion of VEGF, one of the important cytokines that determine the potency of Stempeucel product, showed uniform secretion levels across all the batches (Figure 1). There is consistency in the secretion levels of VEGF from batch to batch, and the value ranges from 2.2 to 3.0 ng/mL per million cells. Results of the three potency-determining parameters of the drug are found to be consistent across the PMS batches and demonstrate the quality attribute of the drug product used in the study to produce efficacy in the patients.

The identity, purity, impurity, strength/potency, sterility, safety, and genetic stability of Stempeucel were assessed in all the seven batches used in the study and showed consistent data in all the batches tested (supplemental online Table 2).

### 3.2 | Phase IV study

Of the 63 patients screened, 50 were enrolled in the study. The CONSORT diagram (Figure 2) shows the number of patients screened and enrolled and who completed the 12-month follow-up.

### 3.3 | Patient characteristics

The demographics and baseline characteristics of the patients are given in Table 1. A total of 50 patients were dosed in the study and were included in safety population. The mean age of the patients was 42 years, and all the patients were male and had at least one ulcer.

### 3.4 | Efficacy results

#### 3.4.1 | Rest pain score

Rest pain scores showed gradual and sustained decrease over the study period of 12 months. The mean ± SD rest pain score reduced from 7.8 ± 1.31 at baseline to 4.4 ± 1.81 at 1 month, 2.2 ± 2.24 at

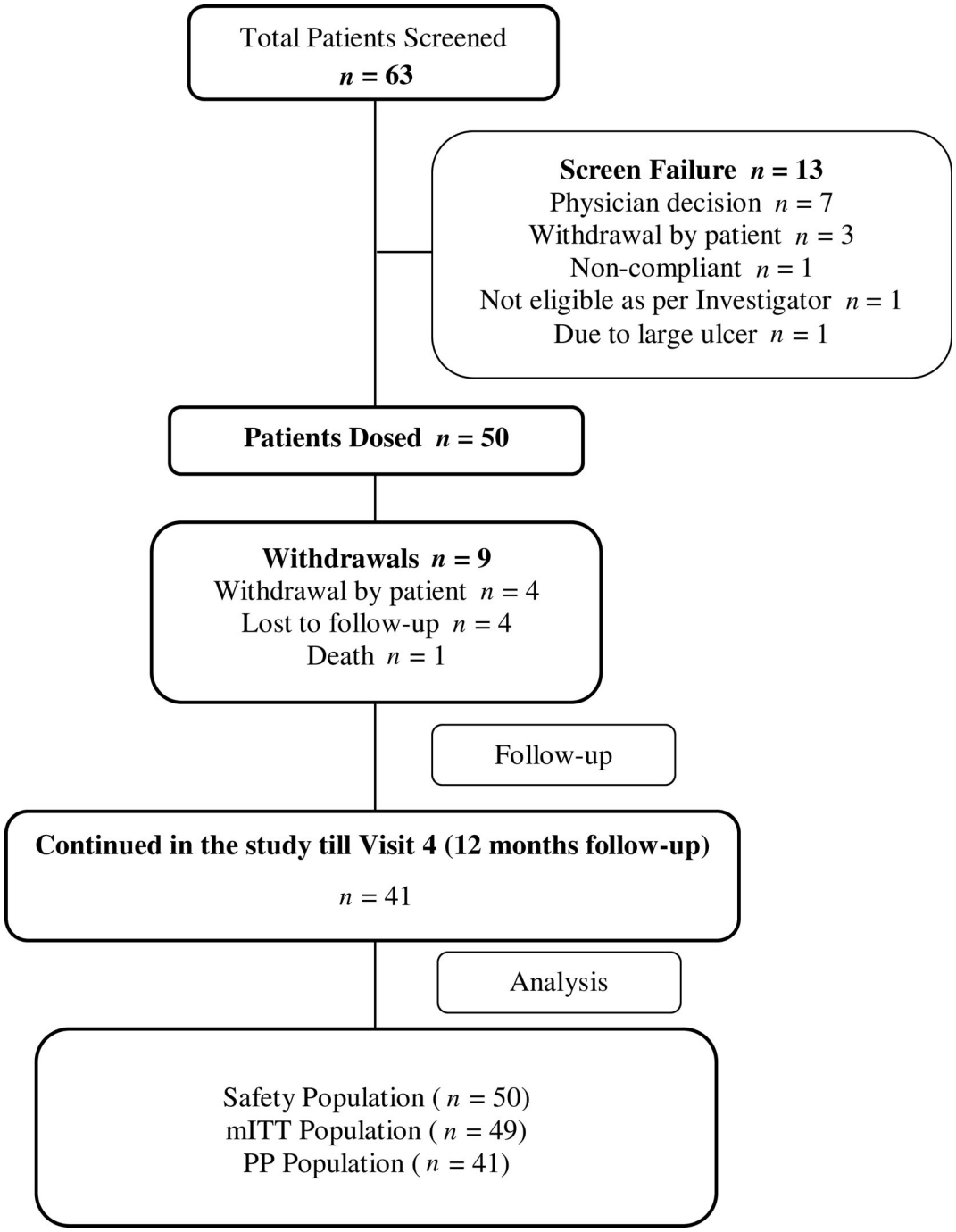


**FIGURE 2** CONSORT diagram showing number of patients enrolled, followed up, and analyzed. Abbreviations: mITT, modified intention to treat; PP, per protocol

6 months, and 1.4 ± 2.36 at 12 months, which was statistically significant ($P < .0001$) as shown in Table 2. The mean ± SD change from baseline was a reduction of 3.5 ± 1.85 at 1 month (43.9% reduction from baseline), 5.6 ± 2.62 at 6 months (70.8% reduction from baseline), and 6.4 ± 2.80 at 12 months (80.5% reduction from baseline).

There was a significant decrease in the rest pain scores by 0.45 unit (SE = 0.032) per month over the period of 12 months ($P < .0001$) compared with baseline as per longitudinal analysis using the GEE method.

### 3.4.2 | Ulcer healing status

Of the 59 ulcers at baseline, 13 ulcers (22%) ($P < .0001$; relative risk [RR], 0.78; 95% confidence interval [CI], 0.65-0.88), 40 ulcers (67.8%) ($P < .0001$; RR, 0.27; 95% CI, 0.16-0.41), and 43 ulcers (72.9%) ($P < .0001$; RR, 0.13; 95% CI, 0.05-0.27) had complete healing at 1 month, 6 months, and 12 months, respectively. The RR suggested the patients were 22%, 73%, and 87% less likely to develop an ulcer at 1 month, 6 months, and 12 months, respectively, compared with baseline. Longitudinal analysis using the GEE method showed significant decreasing pattern in ulcer

healing by 0.42 unit (SE = 0.065) per month over the period of 12 months ($P < .0001$) compared with baseline. Healing of ulcers correlated well with rest pain score. All the subjects who had ulcer healed at 1-year follow-up also had improvement in rest pain score. No new ulcer was observed in the patients during the 12-month follow-up period. The ulcer healing status is summarized in Table 3, and representative images of ulcer healing are shown in Figure 3D.

### 3.4.3 | Ankle systolic pressure

The mean ± SD ASP increased from 56.2 ± 15.56 mmHg at baseline to 79.3 ± 24.57 mmHg at 1 month (visit 2), 90.2 ± 27.22 mmHg at 6 months (visit 3), and 92.3 ± 29.89 mmHg at 12 months (visit 4), which was statistically significant ($P < .0001$) (Table 2). The mean ± SD change from baseline was an increase of 23.6 ± 22.01 mmHg at 1 month (47.1% increase from baseline), 34.4 ± 25.96 mmHg at 6 months (69.3% increase from baseline), and 36.5 ± 27.25 mmHg at 12 months (72.3% increase from baseline).

Longitudinal analysis of ASP assessment showed that there was a significant increasing pattern shown by increase of 2.41 (SE = 0.285) units per month over the period of 12 months ($P < .0001$) compared with baseline.

### 3.4.4 | Ankle brachial pressure index

The ABPI showed gradual increase over the study period of 12 months. The mean ± SD showed increase from 0.44 ± 0.125 at baseline to 0.63 ± 0.222 at 1 month, 0.74 ± 0.218 at 6 months, and 0.75 ± 0.243 at 12 months, which was statistically significant ($P < .0001$) (Table 2). The mean ± SD change from baseline was an increase of 0.19 ± 0.181 at 1 month (46.46% increase from baseline), 0.29 ± 0.211 at 6 months (70.57% increase from baseline), and 0.30 ± 0.226 at 12 months (71.09% increase from baseline).

Longitudinal analysis of ABPI showed that there was a statistically significant increase ($P < .0001$) in the ASP index by 0.04 units (SE = 0.005) per month over a period of 12 months.

The mean (± SE) changes in rest pain, ASP, and ABPI from baseline vs time are depicted in Figure 3A, B, and C, respectively.

**TABLE 1** Demography and baseline characteristics

| Parameter | mITT population ($n = 49$) |
|---|---|
| Age, mean ± SD, years | 42 ± 8.5 |
| Weight, mean ± SD, kg | 59.1 ± 11.08 |
| Gender (male), *n* (%) | 49 (100.0) |
| Height, mean ± SD, cm | 166.4 ± 6.76 |
| Rest pain score, mean ± SD | 7.8 ± 1.31 |
| Ankle systolic pressure, mean ± SD, mmHg | 56.2 ± 15.56 |
| Ankle brachial pressure index, mean ± SD, mmHg | 0.44 ± 0.125 |

Abbreviation: mITT, modified intention to treat.

## 3.5 | Safety results

The AEs were classified by the Medical Dictionary for Regulatory Activities SOC and Preferred Term. A total of 36 TEAEs were reported by 20 (40%) of 50 patients during the study (Table 4). Most of the TEAEs that occurred in the study were assessed as mild to moderate in severity and were resolved. Of these 36 AEs, 17 AEs were mild, 11 AEs were moderate, and 8 AEs were severe in nature. All the AEs were considered to be unrelated to the study drug. The most frequently reported AEs were pyrexia (three patients, 8.4%) and pain in extremity (five patients, 10%). Three patients each underwent major and minor amputation. None of the patients had any clinically significant abnormalities in laboratory parameters or vital signs during the study. A total of nine serious adverse events (SAEs) were reported in the study (supplemental online Table 1). The outcome of one SAE was fatal, and the diagnosis was given as septicemia with septic shock following gangrene of colon and gallbladder.

## 4 | DISCUSSION

In Buerger's disease, no form of therapy is conclusive except for complete abstinence from tobacco, as even a few cigarettes a day may result in progression of the disease. Adjunctive measures like pharmacotherapy and freedom from smoking may help these patients. Additional treatment options include anticoagulants, vasodilators, systemic anti-inflammatory drugs, analgesics, intermittent pneumatic

**TABLE 3** Ulcer healing status

| | Baseline visit | | Month 1/visit 2 | | Month 6/visit 3 | | Month 12/visit 4 | |
|---|---|---|---|---|---|---|---|---|
| Ulcer healing status | Yes, *n* (%) | No, *n* (%) | Yes, *n* (%) | No, *n* (%) | Yes, *n* (%) | No, *n* (%) | Yes, *n* (%) | No, *n* (%) |
| Complete healing | 0 (0.0) | 0 (0.0) | 13 (22.0) | 46 (78.0) | 40 (67.8) | 19 (32.2) | 43 (72.9) | 16 (27.1) |
| Partial healing | 0 (0.0) | 0 (0.0) | 41 (69.5) | 18 (30.5) | 13 (22.0) | 46 (78.0) | 10 (16.9) | 49 (83.1) |
| No healing | 59 (100.0) | 0 (0.0) | 5 (8.5) | 54 (91.5) | 3 (5.1) | 56 (94.9) | 1 (1.7) | 58 (98.3) |
| Missing data | 0 (0.0) | 0 (0.0) | 0 (0.0) | 59 (100.0) | 3 (5.1) | 56 (94.9) | 5 (8.5) | 54 (91.5) |
| *P* value | — | | <.0001 | | <.0001 | | <.0001 | |

**TABLE 2** Rest pain score, ankle systolic pressure, and ABPI

| Descriptive | Rest pain score[a] | | | | Ankle systolic pressure[b] | | | | ABPI[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Visit 1 (baseline) | Month 1/ visit 2 | Month 6/ visit 3 | Month 12/ visit 4 | Visit 1 (baseline) | Month 1/ visit 2 | Month 6/ visit 3 | Month 12/ visit 4 | Visit 1 (baseline) | Month 1/ visit 2 | Month 6/ visit 3 | Month 12/ visit 4 |
| *n* | 49 | 49 | 48 | 48 | 48 | 48 | 47 | 47 | 49 | 49 | 47 | 47 |
| Mean ± SD | 7.8 ± 1.31 | 4.4 ± 1.81 | 2.2 ± 2.24 | 1.4 ± 2.36 | 56.2 ± 15.56 | 79.3 ± 24.57 | 90.2 ± 27.22 | 92.3 ± 29.89 | 0.44 ± 0.125 | 0.63 ± 0.222 | 0.74 ± 0.218 | 0.75 ± 0.243 |
| SE | 0.2 | 0.3 | 0.3 | 0.3 | 2.2 | 3.5 | 4.0 | 4.4 | 0.02 | 0.03 | 0.03 | 0.04 |
| Median | 8.0 | 5.0 | 1.5 | 0.0 | 58.0 | 70.0 | 90.0 | 90.0 | 0.46 | 0.60 | 0.74 | 0.77 |
| Min–max | 4.7-10.0 | 0.5-7.0 | 0.0-9.7 | 0.0-9.0 | 26.0-105.0 | 26.0-130.0 | 26.0-150.0 | 26.0-140.0 | 0.00-0.60 | 0.00-1.00 | 0.00-1.12 | 0.00-1.12 |
| IQR (Q3–Q1) | 1.9 | 2.6 | 2.5 | 2.6 | 15.5 | 40.0 | 46.0 | 47.0 | 0.14 | 0.28 | 0.30 | 0.30 |
| 95% CI | 7.45-8.20 | 3.86-4.90 | 1.54-2.84 | 0.75-2.12 | 51.67-60.71 | 72.16-86.43 | 82.22-98.20 | 83.54-101.10 | 0.403-0.474 | 0.568-0.695 | 0.678-0.806 | 0.678-0.821 |
| *P* value (Shapiro–Wilk) | .0023 | .0119 | <.0001 | <.0001 | .0473 | .1266 | .0952 | .0711 | .0019 | .0206 | .0190 | .0037 |
| *P* value | — | <.0001 | <.0001 | <.0001 | — | <.0001 | <.0001 | <.0001 | — | <.0001 | <.0001 | <.0001 |

Abbreviations: ABPI, ankle brachial pressure index; CI, confidence interval; IQR, interquartile range.

[a]*P* value from Wilcoxon sign rank test.

[b]*P* value from paired *t* test.

compression, spinal cord stimulation, and peripheral sympathectomy, which may improve the symptoms but cannot prevent disease progression.

Iloprost, a prostaglandin analog vasodilator, is also used to manage TAO, and in few clinical trials it has been shown to have significant relief of rest pain, greater healing of ischemic ulcers, and reduction in need for amputation.[15] Another study in 19 patients with TAO concluded that intravenous iloprost therapy did not demonstrate significant changes in wound healing during the treatment and at discharge.[16] In aggregate, the efficacy results shown by iloprost when used for the management of TAO are not completely satisfactory.

Surgical modality of treatment with surgical revascularization is less efficacious because of absence of distal vascular targets.[17] In a few patients, bypass surgery may be considered with severe ischemia and suitable distal target vessels. But these patients undergoing bypass surgery often have poor outcomes with primary patency rates of 41%, 32%, and 30% and secondary patency rates of 54%, 47%, and 39% at 1, 5, and 10 years.[18] The patency rate of the graft in patients with TAO is nearly 50% less with those who continue to smoke after surgery.[19]

The limited options for these patients who had progressed to CLI have generated interest in the field of therapeutic angiogenesis. Effective new biological and angiogenic therapies are the need of the hour. Current literature favors the administration of autologous or allogeneic BMMSCs; the latter are relatively safe and easy to expand ex vivo and appear to be efficacious. Our completed phase II and phase IV study using Stempeucel in Buerger's disease showed that in these no-option patients the cells are capable of alleviating symptoms after standard treatments have failed. Clinical benefits include rapid angiogenesis, reduced inflammation, improvement of ABPI, reduction of rest pain, increased perfusion of ischemic limb, increased healing rates of nonhealing ulcers, and decreased need for amputation.[8,17,20-22]

There are few published reports on clinical trial results using autologous or allogeneic BMMSCs in CLI, but the majority of the reported trials used autologous BMMNCs. The use of allogeneic BMMSCs is advantageous, as they are readily prepared from healthy donors and may be used as an allogeneic, "off-the-shelf" cryopreserved product[23] without HLA matching because of their hypoimmunogenic, immunosuppressive, and immunomodulatory properties. The cell viability, viable cell count, and VEGF potency assay, the key parameters that facilitate the efficiency of cell therapy in clinical trials are assessed in PMS batches. All our analyzed batches have shown good viable cell count of 190 million cells using trypan blue exclusion in the Vi-cell XR automated cell viability analyzer. Further analysis has shown cell viability greater than 90% in all the batches, and these values were obtained exclusively from the 7-AAD flow cytometry experiment. As a potency marker, to analyze the effectiveness of Stempeucel for CLI, we chose a proangiogenic molecule, VEGF. Since CLI is associated with severe impairment and loss of blood vessels, a presence of proangiogenic molecule will aid in the clinical improvement. Also, our phase II clinical trial data showed the formation of new blood vessels in patients injected with Stempeucel.[8]

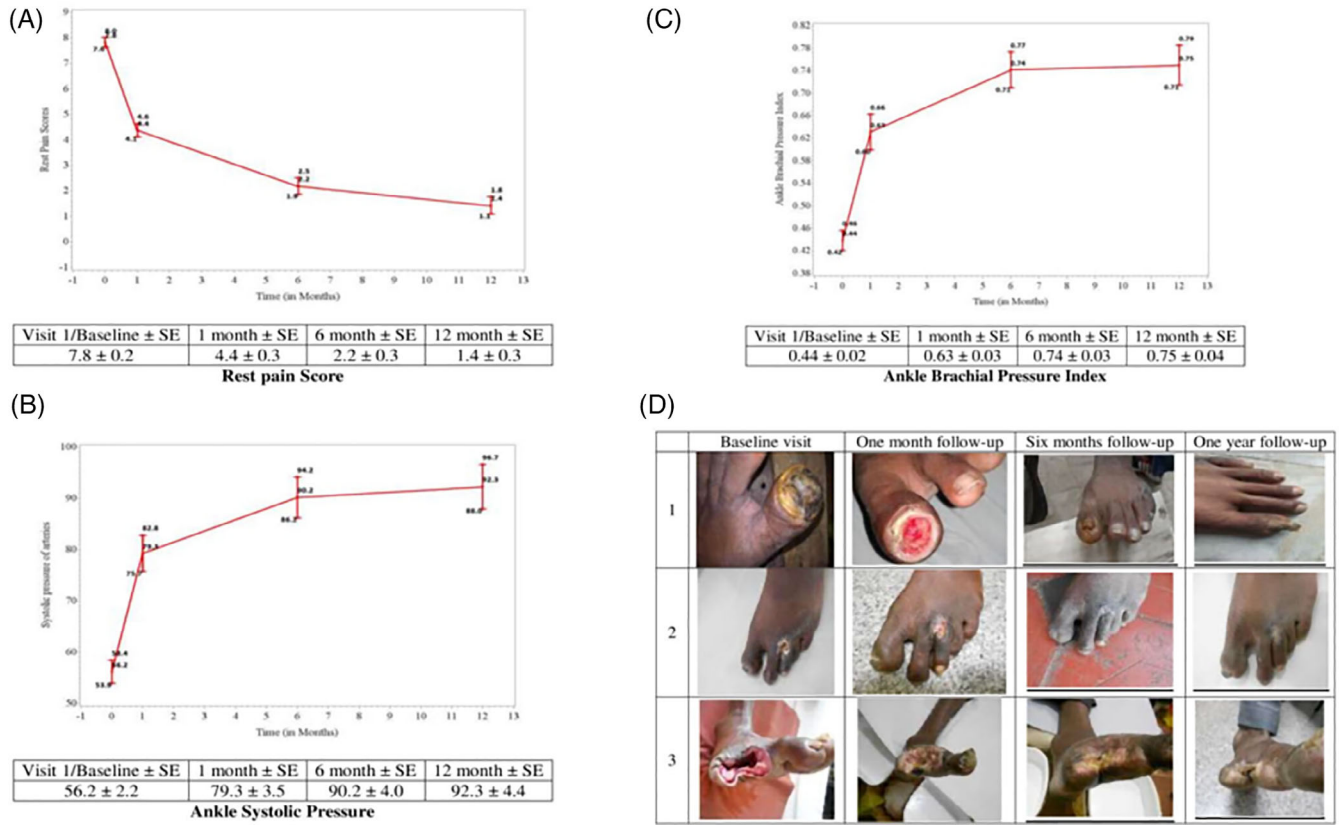


**FIGURE 3** Efficacy score vs time curve and ulcer healing status. (A) Mean (±SE) change in rest pain score from baseline vs time curve. (B) Mean (±SE) change in ankle systolic pressure from baseline vs time curve. (C) Mean (±SE) change in ancle brachial pressure index score from baseline vs time curve. (D) Ulcer healing from baseline across 1 year follow-up

We therefore chose to check the levels of VEGF in every batch as release criteria. Our previously published data showed that varying concentrations of VEGF affect the in vitro proliferation, migration, and tube formation of human umbilical vein endothelial cells. Based on our previous set limit, all our batches of cells uniformly secrete VEGF above 2 ng/mL per million cells.

The postulated mechanism of Stempeucel is through secretion of growth factors, chemokines, and cytokines that are known for their angiogenic, anti-inflammatory, and immunomodulatory functions and also through differentiation into endothelial cells.[24-26] Stempeucel secretes multiple angiogenic factors, of which VEGF, IL-6, and Ang-1 are the most commonly studied, as they are potently angiogenic.[27] Of these factors, VEGF is the most important proangiogenic factor, as it helps to recruit endothelial lineage cells and initiate vascularization. VEGF is believed to be the master regulator of angiogenesis and vasculogenesis and has been shown to play an essential role in physiological and pathological angiogenesis.[25,28,29] We have demonstrated that VEGF is secreted consistently in all manufactured batches at cellular passage 5 corresponding to the Stempeucel product. So, VEGF has been chosen as a surrogate potency marker for this indication. The functional roles of the angiogenic secretome of Stempeucel, such as endothelial migration, proliferation, and tube formation, were further confirmed by in vitro angiogenesis assay in growth factor reduced Matrigel.[11]

The present study was conducted as a phase IV study in a larger patient population to confirm continued efficacy and safety of Stempeucel in patients with CLI due to Buerger's disease. The Central Drugs Standard Control Organization had granted manufacturing and marketing approval for the product Stempeucel after conduct of the phase II dose-finding study for the said indication. Both the phase I/II and phase II studies showed clinical benefit in these patients. Most of the endpoints in the completed studies, like relief of rest pain, ulcer healing, improvement in total walking distance, ABPI, and quality of life, were significant.[8,22]

In the completed phase IV study, intramuscular administration of Stempeucel showed continued long-term efficacy over a period of 12 months' follow-up with a single administration of 2 million cells per kilogram body weight. The reason for continued efficacy of Stempeucel after a single administration was the localized injection of cells at the site of ischemia (infrapopliteal region); these cells predominantly stay localized at the injection site for a minimum duration of 28 days, as shown in our biodistribution studies conducted in a limb ischemia murine model using labeled cells.[30] Stempeucel is capable of secreting a variety of bioactive factors with diverse functional activity, especially those that have been implicated in angiogenesis and anti-inflammatory properties, such as VEGF, TGF-β, HGF, angiopoietin 1 and 2, IL-8, Indoleamine 2,3-DiOxygenase (IDO), and Prostaglandin E2 (PGE2), among others. This may result in continued improvement as seen in these patients. The efficacy result showed statistically significant

**TABLE 4** Summary of treatment-emergent adverse events

| SOC, adverse events by PT | Patients ($n = 50$), $n$ (%) | Events ($n = 36$), $n$ (%) |
|---|---|---|
| Gastrointestinal disorders | | |
| Constipation | 1 (2.0) | 1 (2.8) |
| Diarrhea | 1 (2.0) | 1 (2.8) |
| Vomiting | 1 (2.0) | 1 (2.8) |
| General disorders and administration site conditions | | |
| Pain | 2 (4.0) | 3 (8.3) |
| Pyrexia | 3 (6.0) | 3 (8.3) |
| Infections and infestations | | |
| Infected skin ulcer | 2 (4.0) | 2 (5.6) |
| Wound infection | 3 (6.0) | 3 (8.3) |
| Injury, poisoning and procedural complications: Injury | 1 (2.0) | 1 (2.8) |
| Metabolism and nutrition disorders | | |
| Hyperglycemia | 1 (2.0) | 1 (2.8) |
| Hypertriglyceridemia | 1 (2.0) | 1 (2.8) |
| Musculoskeletal and connective tissue disorders: Pain in extremity | 2 (4.0) | 5 (13.9) |
| Renal and urinary disorders: Urinary retention | 1 (2.0) | 1 (2.8) |
| Respiratory, thoracic, and mediastinal disorders: Cough | 2 (4.0) | 2 (5.6) |
| Surgical and medical procedures | | |
| Foot amputation | 1 (2.0) | 1 (2.8) |
| Leg amputation | 3 (6.0) | 3 (8.3) |
| Thrombectomy | 1 (2.0) | 2 (5.6) |
| Toe amputation | 1 (2.0) | 1 (2.8) |
| Vascular disorders | | |
| Peripheral ischemia | 1 (2.0) | 1 (2.8) |
| Thrombophlebitis | 1 (2.0) | 1 (2.8) |
| Dry gangrene | 2 (4.0) | 2 (5.6) |
| Total | 20 (40.0) | 36 (100.0) |

Abbreviations: PT, preferred term; SOC, system organ class.

improvement in rest pain, acceleration of ulcer healing including complete healing of ulcer, and increase in ASP and ABPI (all $P < .0001$) as compared with the baseline visit. Rest pain reduced during the 1-year follow-up period to 43.9%, 70.8%, and 80.5% from baseline at the 1, 6, and 12 months' follow-up period, respectively. This result clearly demonstrates that Stempeucel indeed relieved the rest pain, which could not be ameliorated by the existing drugs, and thereby stopped or reduced the amount of drug intake. Similarly, ABPI increased during the 1-year follow-up period to 46.46%, 70.57%, and 71.09% from baseline at the 1, 6, and 12 months' follow-up period, respectively. The mean increase in the absolute values was 0.19, 0.29, and 0.30 units at 1, 6, and 12 months, respectively. It can be seen that the rise of ABPI plateaued at 6 months and thereafter the increase was minimal. ABPI remains an invaluable tool in assessment of vascular patients, especially those with atypical presentation, and in determining the success or otherwise of different therapies in these patients. ABPI is also used to define any progress in the disease process, as a decrease in ABPI of 0.15 is associated with an increased risk for bypass interventions (2.5-fold) and symptom progression (1.8-fold). ABPI is a global estimator of whole limb perfusion, and an increase of 0.10 and 0.15 units in the affected limb predicts no residual stenosis in >50% of cases with sensitivities of 79% and 67% and specificities of 92% and 100%, respectively.[31] In our current study we have demonstrated an increase in mean ABPI from 0.44 units at baseline to 0.75 units at 1 year follow-up (mean increase of 0.30 units), which indirectly predicts that the overall perfusion of the affected limb has improved, which is clinically beneficial to these patients. Hence, ABPI may be an important parameter that gives indirect evidence of neoangiogenesis, increased ulcer healing, and relief of rest pain in these patients who are young and have few cardiovascular risk factors.

CLI due to Buerger's disease is a progressive disease that results in tissue loss, gangrene, and ultimately amputation of the affected limb regardless of treatment. Few studies have evaluated the long-term outcomes including major and minor amputations and survival in these patients. In one study of 110 patients, at 25 years of follow-up, the survival rate was 84%, and the rate of amputation was 43% with 12% being major amputations.[18] In another study of 111 patients followed up for a mean of 15 years, the risk of major amputation was 11%, 21%, and 23% at 5, 10, and 20 years, respectively.[32] Furthermore, based on reported studies,[33] at the end of a 5-year follow-up period, one quarter of patients are likely to have had an amputation. After 10 years, the risk of amputation is up to 45%. The risk of major amputation remains between 4.4% and 11.8%. Hence, to evaluate amputation or amputation-free survival in patients with TAO will require a larger patient population but will also require long-duration trials with a follow-up for 5 to 10 years, which remains a challenge in this orphan indication. When comparing the risk of major amputation for Stempeucel with other forms of treatment, for example, VEFG, FGF, or CD34+ cells, the rate of major amputation varies between 22% and 36% at a maximum follow-up of 12 months in these therapies.[34-36] In this trial, 12% of the patients had major and minor amputations, 6% of patients had major amputation, and survival was 98%. Three patients (6%) had major amputation due to the advanced nature of the disease; these were no-option patients who were not responding to current therapeutic options.

The safety of MSC therapy is important, and any risk of AEs could represent a significant barrier to MSCs' use in clinical practice. The potential risks include neoplastic proliferation, susceptibility to infection due to MSCs' immunomodulatory properties, embolism of cells, and zoonosis associated with culture reagents.[37] In different systemic reviews analyzing the safety profile of MSCs, fever has been identified as the only AE that is significantly associated with MSC therapy.[38,39] There is no association between MSC treatment and the development of nonfever acute infusional toxicity, infection or malignancy, and the development of thrombotic or thromboembolic events. In this study, 36 TEAEs were reported by 20 (40%) of 50 patients. Most of the AEs were mild to moderate in nature. A total of nine SAEs were reported in the study. Of the nine SAEs, eight SAEs were severe, and one was moderate in nature.

Patients recovered from five SAEs with sequelae and three SAEs without sequelae, and one SAE was fatal. All AEs and SAEs were not related to the Stempeucel and were due to progress of the disease.

## 5 | CONCLUSION

Our data demonstrate that cellular medicine, Stempeucel in patients with CLI due to Buerger's disease, has a significant improvement in rest pain score, ulcer healing, and increase of ABPI and ASP from baseline across visits until 1 year of follow-up. Furthermore, there were no cases showing development of SAEs are related to Stempeucel. In conclusion, our findings from this PMS phase IV study demonstrate that intramuscular administration of Stempeucel continues to be a safe, tolerable, and effective alternative to achieve therapeutic angiogenesis in no-option patients with CLI due to Buerger's disease.

### CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest.

### AUTHOR CONTRIBUTIONS

P.K.G.: conception/design, administrative support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; S.D., S.K., M.N., S.C.D., S.S.M., A.D., R.R., M.R., A.B.: provision of study material or patients, collection and/or assembly of data; S.P.: administrative support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; N.S.R., P.V., M.C.: administrative support, final approval of manuscript; C.T., K.V.P., H.B.: administrative support, provision of study material or patients, collection and/or assembly of data, final approval of manuscript; J.A.: administrative support, provision of study material or patients, final approval of manuscript; K.U.: administrative support, collection and/or assembly of data, final approval of manuscript.

### DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### REFERENCES

1. Arkkila PET. Thromboangiitis obliterans (Buerger's disease). *Orphanet J Rare Dis*. 2006;1:14.
2. López de Maturana I, Rodriguez J, González C, et al. Chronic ulcers in thromboangiitis obliterans (Buerger's disease): updating epidemiology, physiopathology, and bosentan—a novel strategy of therapy. *Ulcers*. 2013;2013:230780.
3. Piazza G, Creager MA. Thromboangiitis obliterans. *Circulation*. 2010; 121:1858-1861.
4. Uhl SM, Matsushita MM, Shionoya SM. Urinary cotinine measurement in patients with Buerger's disease—effects of active and passive smoking on the disease process. *J Vasc Surg*. 1991;14:53-58.
5. Iwai T, Inoue Y, Umeda M, et al. Oral bacteria in the occluded arteries of patients with Buerger disease. *J Vasc Surg*. 2005;42:107-115.
6. Olin JW, Young JR, Graor RA, et al. The changing clinical spectrum of thromboangiitis obliterans (Buerger's disease). *Circulation*. 1990;82-(suppl 5):3-8.
7. Olin JW. Thromboangiitis obliterans (Buerger's disease). *N Engl J Med*. 2000;343:864-869.
8. Gupta PK, Krishna M, Chullikana A, et al. Administration of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells in critical limb ischemia due to Buerger's disease: phase II study report suggests clinical efficacy. *STEM CELLS TRANSLATIONAL MEDICINE*. 2017;6:689-699.
9. Wester T, Jørgensen JJ, Stranden E, et al. Treatment with autologous bone marrow mononuclear cells in patients with critical lower limb ischaemia. A pilot study. *Scand J Surg*. 2008;97:56-62.
10. Das AK, Abdullah BJJB, Dhillon SS, et al. Intra-arterial allogeneic mesenchymal stem cells for critical limb ischemia are safe and efficacious: report of a phase I study. *World J Surg*. 2013;37:915-922.
11. Thej C, Ramadasse B, Walvekar A, Majumdar AS, Balasubramanian S. Development of a surrogate potency assay to determine the angiogenic activity of Stempeucel, a pooled, ex-vivo expanded, allogeneic human bone marrow mesenchymal stromal cell product. *Stem Cell Res Ther*. 2017;8:47.
12. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *STEM CELLS*. 2007;25:2648-2659.
13. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42:121-130.
14. Wang M. Generalized estimating equations in longitudinal data analysis: a review and recent developments. *Adv Stat*. 2014;2014:303728.
15. Fiessinger JN, Schäfer M. Trial of iloprost versus aspirin treatment for critical limb ischaemia of thromboangiitis obliterans. *Lancet*. 1990; 335:555-557.
16. Afsharfard A, Mozaffar M, Malekpour F, Beigiboroojeni A, Rezaee M. The wound healing effects of Iloprost in patients with Buerger's disease: claudication and prevention of major amputations. *Iran Red Crescent Med J*. 2011;13:420-423.
17. Dash BC, Peyvandi H, Duan K, et al. Stem cell therapy for thromboangiitis obliterans (Buerger's disease). *Processes*. 2020;8:1408.
18. Ohta T, Ishioashi H, Hosaka M, Sugimoto I. Clinical and social consequences of Buerger disease. *J Vasc Surg*. 2004;39:176-180.
19. Sasajima T, Kubo Y, Inaba M, Goh K, Azuma N. Role of infrainguinal bypass in Buerger's disease: an eighteen-year experience. *Eur J Vasc Endovasc Surg*. 1997;13:186-192.
20. Fazeli B, Dadgar Moghadam M, Niroumand S. How to treat a patient with thromboangiitis obliterans: a systematic review. *Ann Vasc Surg*. 2018;49:219-228.
21. Ra JC, Jeong EC, Kang SK, Lee SJ, Choi KH. A prospective, non-randomized, 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. *Cell Med*. 2017;9:87-102.
22. Gupta PK, Chullikana A, Parakh R, et al. A double blind randomized placebo controlled phase I/II study assessing the safety and efficacy of allogeneic bone marrow derived mesenchymal stem cell in critical limb ischemia. *J Transl Med*. 2013;11:143.
23. Hare JM, Traverse JH, Henry TD, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:2277-2786.
24. Au P, Tam J, Fukumura D, Jain RK. Bone marrow derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. *Blood*. 2008;111:4551-4558.
25. Cobellis G, Maione C, Botti C, et al. Beneficial effects of VEGF secreted from stromal cells in supporting endothelial cell functions:

therapeutic implications for critical limb ischemia. *Cell Transplant*. 2010;19:1425-1437.
26. Ranganath SH, Levy O, Inamdar MS, Karp JM. Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease. *Cell Stem Cell*. 2012;10:244-258.
27. Balasubramanian S, Thej C, Walvekar A, et al. Evaluation of the secretome profile and functional characteristics of human bone marrow mesenchymal stromal cells-derived conditioned medium suggest potential for skin rejuvenation. *J Cosmet Dermatological Sci Appl*. 2017;7:99-117.
28. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol*. 1995;11:73-91.
29. Ferrara N. Molecular and biological properties of vascular endothelial growth factor. *J Mol Med (Berl)*. 1999;77:527-543.
30. Thej C, Balasubramanian S, Rengasamy M, et al. Human bone marrow-derived, pooled, allogeneic mesenchymal stromal cells manufactured from multiple donors at different times show comparable biological functions in vitro, and in vivo to repair limb ischemia. *Stem Cell Res Ther*. 2021;12:279.
31. Decrinis M, Doder S, Stark G, Pilger E. A prospective evaluation of sensitivity and specificity of the ankle/brachial index in the follow-up of superficial femoral artery occlusions treated by angioplasty. *Clin Investig*. 1994;72:592-597.
32. Cooper LT, Tse TS, Mikhail MA, McBane RD, Stanson AW, Ballman KV. Long-term survival and amputation risk in thromboangiitis obliterans (Buerger's disease). *J Am Coll Cardiol*. 2004;44:2410-2411.
33. Puéchal X, Fiessinger JN. Thromboangiitis obliterans or Buerger's disease: challenges for the rheumatologist. *Rheumatology*. 2007;46:192-199.
34. Skóra J, Pupka A, Janczak D, et al. Combined autologous bone marrow mononuclear cell and gene therapy as the last resort for patients with critical limb ischemia. *Arch Med Sci*. 2015;11:325-331.
35. Losordo DW, Kibbe MR, Mendelsohn F, et al. A randomized, controlled pilot study of autologous CD34+ cell therapy for critical limb ischemia. *Circ Cardiovasc Interv*. 2012;5:821-830.
36. Belch J, Hiatt WR, Baumgartner I, et al. Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia. *Lancet*. 2011;377:1929-1937.
37. Prockop DJ, Brenner M, Fibbe WE, et al. Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy*. 2010;12:576-578.
38. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 2012;7:e47559.
39. Thompson M, Mei SHJ, Wolfe D, et al. Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: an updated systematic review and meta-analysis. *EClinicalMedicine*. 2020;19:100249.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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