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Safety of autologous bone marrow-derived mesenchymal stem cells in erectile dysfunction: an open-label phase 1 clinical trial

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ABSTRACT

Background aims: The efficacy of phosphodiesterase type 5 inhibitors (PDE5Is), which are commonly used to treat erectile dysfunction (ED), is not satisfactory in patients with denervation of the cavernous nerve due to pelvic surgeries and diabetes mellitus (DM). Pre-clinical studies using bone marrow-derived mesenchymal stem cells (BMSCs) to treat ED have shown promising results. The authors conducted a phase 1 clinical trial with autologous BMSCs in patients with ED due to radical prostatectomy or DM.
Methods: Ten patients (five with post-prostatectomy ED and five with DM-associated ED) who could not perform sexual activity despite taking the maximum dose of a PDE5I were enrolled. The brief clinical trial protocol was registered with the US National Institutes of Health on ClinicalTrials.gov (NCT02344849). The primary outcome was the safety of stem cell therapy, and the secondary outcome was the improvement of erectile function.
Results: Of the 13 patients screened, 10 were registered in the clinical trial and received autologous BMSCs and nine completed the clinical trial. One patient with post-prostatectomy ED experienced two treatment-emergent adverse events (TEAEs) (pyrexia and back pain), and two patients with DM-associated ED experienced a total of five TEAEs (one case each of viral upper respiratory tract infection, prostatitis and pruritus and two cases of hyperglycemia). Of these patients, one with DM-associated ED experienced two serious TEAEs (two instances of hyperglycemia). All TEAEs were considered not to be related to autologous BMSC therapy. In addition, no clinical significance was identified related to other safety measures, such as laboratory tests and vital signs. The mean International Index of Erectile Function score increased significantly at 1 month versus baseline (24.9 versus 18.1, $P = 0.0222$).
Conclusions: This phase 1 clinical trial confirmed the safety and potential efficacy of autologous BMSC therapy in patients with ED. The authors' results need to be confirmed by a phase 2 clinical trial.

Introduction

Erectile dysfunction (ED) is defined as the inability to obtain or maintain an erection sufficient for satisfactory sexual intercourse [1]. Data from the US National Health and Nutrition Examination Survey show that the overall prevalence of ED is 18.4% in men aged ≥20 years, with a higher prevalence among older men [2]. Phosphodiesterase type 5 inhibitors (PDE5Is) are widely used in ED treatment because of their excellent efficacy and safety profiles. However, 30–35% of patients with ED fail to respond to PDE5Is [3]. The response rate for PDE5Is is lower in some patient groups, such as those who have undergone pelvic surgeries or have diabetes mellitus (DM), than in the general population with ED [4]. These conditions reduce the

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bioavailability of nitric oxide as a result of degeneration of the nitrergic nerves that supply the penile corpora cavernosa and vasculature [5,6]. A sound nitric oxide supply from the nerves and endothelium is key to ensuring the efficacy of PDE5Is. Anatomical injury of the pelvic and cavernous nerves is also a common reason for ED after pelvic surgeries.

Many studies have shown that treatment with various cultured and uncultured cells can restore erectile function in animal models of cavernous nerve injury and DM [7]. Based on these pre-clinical data, several human clinical trials using various regenerative cells have been reported for ED treatment [8–14]. Some clinical trials have used uncultured regenerative cells from bone marrow or adipose tissue [10,11]. Uncultured cells are unfavorable for multiple injections because cell viability is decreased during freezing and thawing [15]. Some clinical trials of cultured regenerative cells have used allogeneic stem cells [8,9,12]. Although stem cells are minimally immunogenic, major histocompatibility complex can be re-expressed if allogeneic stem cells are repeatedly administered [16].

Therefore, the authors focused on autologous cultured cells and confirmed the efficacy and safety of human bone marrow-derived mesenchymal stem cell (BMSC) therapy in a pre-clinical study [17–19]. Herein the authors report the safety and preliminary efficacy outcomes from a phase 1 clinical trial using autologous BMSCs for the treatment of ED in 10 patients with ED due to radical prostatectomy or DM.

Methods

Study design

This open-label, single-center, phase 1 clinical trial was designed to evaluate the safety and potential efficacy of autologous BMSC therapy in patients with ED. The clinical trial protocol was authorized by the Ministry of Food and Drug Safety, Republic of Korea (30328), and approved by the institutional review board of Asan Medical Center, Seoul, Republic of Korea (2015–0219), and conformed to the tenets of the Declaration of Helsinki. The brief clinical trial protocol was registered with the US National Institutes of Health on ClinicalTrials.gov (NCT02344849). The clinical trial protocol and schedule are outlined in Figure 1 and Table 1. The clinical trial protocol was designed based on results of a previous pre-clinical study [18].

The authors aimed to recruit 10 patients (five with post-prostatectomy ED and five with DM-associated ED) in whom erectile function was unlikely to recover with PDE5I treatment. Potential candidates for post-prostatectomy ED included those whose preoperative erectile function was normal and whose prostate-specific antigen (PSA) was <0.04 ng/mL for ≥24 months after surgery. Potential candidates for DM-associated ED included those who suffered from diabetic ED that did not respond to PDE5I treatment and who were considering intracavernous injection therapy. Potential candidates provided written informed consent before participating in the screening. Screening tests were conducted after potential candidates agreed in writing to participate in the clinical trial. The patients who were eligible based on the inclusion and exclusion criteria were registered in the clinical trial, and bone marrow harvesting was performed. Autologous BMSCs were injected into the corpus cavernosum once 1 month after harvesting. The safety and efficacy of the treatment were assessed with patient visits to a hospital at 1 month, 3 months, 6 months, 9 months and 12 months after the injection of autologous BMSCs.

Screening tests and eligibility criteria

Baseline evaluations included demographics, past history, vital signs, physical examination, chest x-rays, electrocardiography, laboratory tests, tumor markers, serologic tests, International Index of Erectile Function (IIEF) score, Sexual Encounter Profile (SEP) 2 and 3 scores and concomitant medication. Laboratory tests included assessments of white blood cell counts with differential, red blood cell counts, hemoglobin, hematocrit, platelet counts, hemoglobin A1c (HbA1c), testosterone, calcium, inorganic phosphorus, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, cholesterol, glucose, blood urea nitrogen, total bilirubin, uric acid, creatinine, sodium, potassium, chloride and urinalysis. Tumor markers included PSA, carcinoembryonic antigen and alpha-fetoprotein. Serologic tests included HIV, hepatitis B virus, hepatitis C virus and syphilis testing.

The patients eligible for inclusion in the study were men aged ≥20 years who could not perform sexual activity (≥4 times) with proper sexual stimulation despite taking the maximum dose of PDE5Is within the previous 8 weeks and who had a consistent partner who was willing to engage in sexual activity ≥2 times per month during the study. The patients had moderate to severe ED (≤16) based on the IIEF Erectile Function domain score [20]. In addition, patients with post-prostatectomy ED included those who had normal preoperative erectile function, were interested in restoring erectile function after surgery, had a preoperative PSA level <10 ng/mL, had a pathological Gleason score ≤7, had pathological stage ≤T2c and had a PSA level <0.04 ng/mL for ≥24 months after surgery without additional therapy. Patients with DM-associated ED included those who were interested in restoring erectile function, agreed to participate in clinical trial and had 6.5≤ HbA1c level ≤10%.

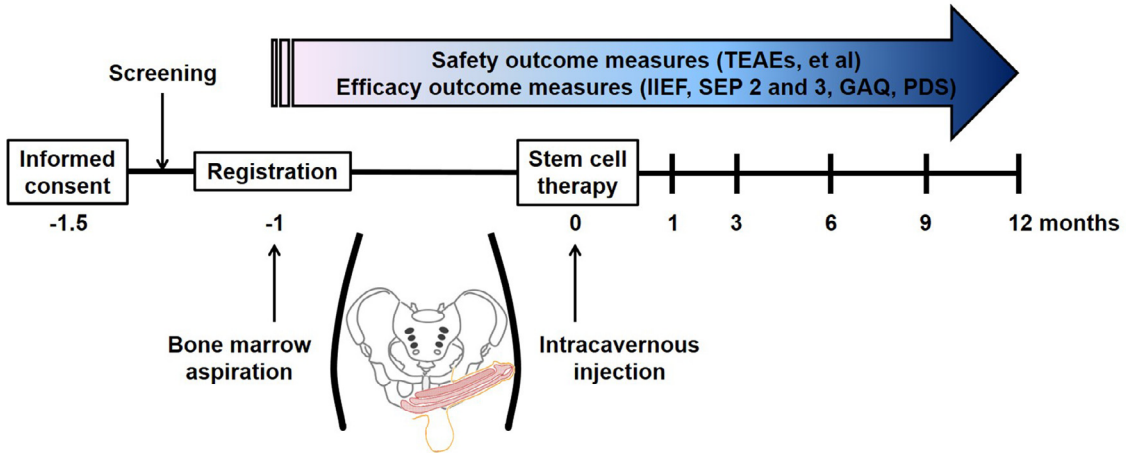

Figure 1. Clinical trial protocol. (Color version of figure is available online).

Table 1
Clinical trial schedule.

	Screening	Registration	Stem cell therapy	Follow-up				
Visit	1	2	3	4	5	6	7	8
Visiting date, months	−1.5	−1	0	1	3	6	9	12
Window, days	± 15	± 7		± 7	± 7	± 7	± 14	± 14
Informed consent	√							
Demographics[a]	√							
Past history	√							
Vital signs[b]	√	√	√	√	√	√	√	√
Physical examination	√	√	√	√	√	√	√	√
Chest x-ray	√					√		√
Electrocardiography	√							
Laboratory tests[c]	√		√	√	√	√	√	√
PSA[d]	√				√	√	√	√
CEA, AFP	√							√
Serologic tests	√							
Bone marrow aspiration[e]		√						
Stem cell injection[f]			√					
Adverse events[h]		√	√	√	√	√	√	√
IIEF	√			√	√	√	√	√
SEP 2 and 3	√			√	√	√	√	√
GAQ				√	√	√	√	√
PDS		√				√		√
Concomitant medication[g]	√	√	√	√	√	√	√	√
TEAEs[h]		√	√	√	√	√	√	√
National Cancer Screening Program[i]								√

AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen.
[a] Date of birth, weight, height.
[b] Body temperature, blood pressure (systolic/diastolic) and pulse rate.
[c] Hematologic tests (white blood cell count, red blood cell count, hemoglobin, hematocrit, platelets, white blood cell differential and HbA1c), chemistry tests (testosterone at visit one, calcium, inorganic phosphorus, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, cholesterol, glucose, blood urea nitrogen, total bilirubin, uric acid, creatinine, sodium, potassium and chloride) and urinalysis (specific gravity, pH, white blood cells, protein, glucose, blood, ketones, bilirubin, urobilinogen and nitrite).
[d] Measured at visits one, five, six, seven and eight in patients with post-prostatectomy ED and at visits one, six and eight in patients with DM-associated ED.
[e] Performed after performing PDS.
[f] Injected into the corpus cavernosum at 1 month after bone marrow aspiration.
[g] Recorded from visit two to 3 months after stem cell injection. However, medications used to treat TEAEs were recorded until recovery or stabilization of TEAEs. Oral ED drugs were investigated from 8 weeks prior to screening to the end of the clinical trial.
[h] Investigated from visit two to 3 months after stem cell injection. After 3 months, only current adverse events or those related to the clinical trial drugs were recorded. Serious adverse events were investigated until the end of the clinical trial. Serious adverse events in progress at the end of the clinical trial were followed until recovery or stabilization.
[i] Candidates were invited to participate in the National Cancer Screening Program, and the results of cancer screening were collected at visit eight.

Patients were excluded if they had a history of bone marrow disorders; had an aspartate aminotransferase/alanine aminotransferase >3 times the upper limit of normal or a creatinine >1.5 times the upper limit of normal; had a history of hypersensitivity to gentamicin; suffered from severe cardiovascular disease (angina, arrhythmia, cardiac failure, stroke), kidney failure or respiratory failure; had a positive test for HIV, hepatitis B virus, hepatitis C virus or syphilis; had a PSA, carcinoembryonic antigen or alpha-fetoprotein level outside of the normal range; had a history of cancer in the previous 5 years (except prostate cancer); suffered from uncontrolled hypertension or hypotension (systolic blood pressure >170 or <90 mmHg, diastolic pressure >100 or <50 mmHg); had an HbA1c level >10%; were receiving anticoagulant treatment; suffered from a severe infectious disease; had a testosterone level <200 ng/dL; had a penile implant or were willing to have one; had morphological changes of the penis; had a partner who was trying to conceive during the clinical trial period; were unwilling to participate in the clinical trial; had participated in other clinical trials in the previous 30 days; were unable to comply with the protocol; or were ineligible to participate in the clinical trial as determined by the investigator.

Isolation and culture of autologous BMSCs

All manufacturing and product testing procedures for the generation of clinical-grade autologous BMSCs (Cellgram-ED; Pharmicell Co, Ltd, Seongnam, Republic of Korea) were performed under Good Manufacturing Practice conditions. Approximately 10 mL of bone marrow was obtained from the posterior superior iliac crest of patients under local anesthesia. Mononuclear cells were separated from the bone marrow by density gradient centrifugation (Histopaque-1077; Sigma-Aldrich, St Louis, MO, USA) and washed with phosphate-buffered saline. Cells were resuspended in low-glucose Dulbecco's Modified Eagle's Medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco) and 20 μg/mL gentamicin (Gibco) and plated at a density of 1.3–2.6 × 10^5 cells/cm^2 in 75-cm^2 flasks (Thermo Fisher Scientific Inc, Waltham, MA, USA). Cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide. After 5–7 days, the nonadherent cells were removed by replacing the medium, and the adherent cells were cultured for another 2–3 days. Colonized cells were detached with trypsin containing ethylenediaminetetraacetic acid (Gibco) and replated at a density of 4.0–5.7 × 10^3 cells/cm^2 in 175-cm^2 flasks (Thermo Fisher Scientific Inc). When they reached 70–80% confluency, cells were serially subcultured up to passage four or five for injection.

The criteria for release of autologous BMSCs for clinical use included the absence of microbial contamination (bacteria, fungus, mycoplasma and endotoxin), viability greater than 70% when assessed using a Trypan Blue exclusion assay and immune phenotyping by flow cytometry analysis proving the expression of CD73 and CD105 surface molecules (>85%) and the absence of CD14, CD34 and CD45 (<3%).

Autologous BMSC injection

On the day of injection, autologous BMSCs were harvested using trypsin; washed twice with phosphate-buffered saline and once with multiple electrolytes injection, type 1, USP (Plasma Solution A; CJ HealthCare, Seoul, Republic of Korea); and resuspended to a final concentration of 1.5×10^7 cells/mL in 2 mL of Plasma Solution A (total 3×10^7 cells). Cellgram-ED is a prefilled syringe in which 3×10^7 autologous BMSCs are suspended with 2 mL of Plasma Solution A.

A tourniquet was applied at the base of the penis to induce an artificial erection that ensured uniform distribution of Cellgram-ED throughout the corpus cavernosum. A 21-gauge needle was installed into a prefilled syringe. Cellgram-ED was lightly tapped with the fingers or shaken just before injection, allowing the cell sediment and suspending agents to mix evenly. Cellgram-ED was injected slowly into the corpus cavernosum on the right or left side, avoiding major structures such as nerves, blood vessels and the urethra. The tourniquet was maintained for 30 min after injection. After monitoring for adverse events for ≥2 h after injection, the patient returned home if no serious adverse events were noted.

Outcome measures and follow-up

The primary endpoint was the occurrence of treatment-emergent adverse events (TEAEs) within 12 months after the injection. All TEAEs that occurred during the clinical trial were standardized according to the system organ class and preferred term of version 20.0 of the Medical Dictionary for Regulatory Activities (MedDRA) (MedDRA MSSO, McLean, VA, USA). TEAEs were reported with regard to their seriousness, severity and causal relationship with the clinical trial drug as the number and percentage of subjects. Other safety outcome measures included laboratory tests, vital signs, physical examination findings and other safety-related items (chest x-rays and tumor markers). For laboratory tests and other safety-related items, each item was classified as normal, abnormal non-clinical significance or abnormal clinical significance (CS), and for physical examination findings, abnormal CS was presented.

The efficacy outcome measures included change in the IIEF score at 1 month, 3 months, 6 months, 9 months and 12 months after the injection of autologous BMSCs. The IIEF is a self-reported erectile function assessment questionnaire consisting of 15 questions in five domains: erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction [21]. Other efficacy outcome measures included the SEP 2 and 3 and Global Assessment Question (GAQ) scores at 1 month, 3 months, 6 months, 9 months and 12 months and the penile Doppler sonography (PDS) at 6 and 12 months after the injection of autologous BMSCs. The SEP 2 and 3 are questionnaires that are completed after each sexual intercourse attempt and contain the following question: were you able to insert your penis into your partner's vagina, and did your erection last long enough for you to have successful intercourse? [22]. The GAQ 1 and 2 are patient-reported outcomes that contain the following questions: has the treatment you have been taking improved your erectile function? If yes, has the treatment improved your ability to engage in sexual activity? The authors also measured the peak systolic velocity (PSV) and end-diastolic velocity (EDV) in the cavernosal artery before and after intracavernous injection of a vasodilator (0.2 mL of Standro, 1.2 μg of alprostadil plus 3.528 mg of papaverine hydrochloride plus 0.12 mg of phentolamine mesylate; Shin Poong Pharma Co, Ltd, Seoul, Republic of Korea). In addition, the resistive index (RI) was computed with the following formula: RI = (PSV − EDV) / PSV. It was recommended that patients continue PDE5Is during the clinical trial.

Statistical analysis

Quantitative data are expressed as numbers, mean ± standard deviation and median (range). Categorical data are expressed as frequencies and ratios (%). All safety analyses were performed on the safety set. All efficacy analyses were performed on the full analysis set. For the mean change from baseline by visit (laboratory tests, vital signs, IIEF score and PDS), Student's paired *t*-test (when satisfied) or Wilcoxon signed-rank test (when not satisfied) was performed according to the satisfaction of the normality assumption. The SEP 2 and 3 questionnaires were compared at baseline (screening) and after cell treatment with McNemar test. Unless otherwise specified, all statistical tests were performed with two-sided tests at a significance level of 5%, and 95% confidence intervals are reported for both sides. Statistical analysis was performed using SAS 9.4 (SAS institute, Cary, NC, USA).

Results

Demographic and baseline characteristics

Of the 13 patients screened, 10 were registered in the clinical trial and received the autologous BMSC treatment. Of the 10 patients registered, one withdrew consent and nine completed the clinical trial (Figure 2).

The demographic and baseline characteristics of registered patients are outlined in Table 2. The overall mean age was 62.0 ± 13.0 years and 67.8 ± 9.1 years and 57.0 ± 14.3 years in patients with post-prostatectomy ED and those with DM-associated ED, respectively. The overall mean duration of ED was 49.0 ± 44.8 months and 59.8 ± 35.7 months and 32.8 ± 57.5 months in patients with post-prostatectomy ED and those with DM-associated ED, respectively.

Safety

During the clinical trial, three out of 10 patients experienced seven TEAEs. One patient with post-prostatectomy ED experienced two TEAEs (pyrexia and back pain), and two patients with DM-associated ED experienced a total of five TEAEs (viral upper respiratory tract infection, prostatitis, pruritus and two cases of hyperglycemia). Of these patients, one with DM-associated ED experienced two serious TEAEs (two episodes of hyperglycemia). Five of seven TEAEs were mild and two were moderate. Two moderate TEAEs were reported in patients with DM-associated ED. All TEAEs were considered not to be related to autologous BMSC therapy. No TEAEs resulting in death or dropout were reported during the clinical trial (Table 3). After the injection of autologous BMSCS, no abnormal CS was observed for other safety measures, such as laboratory tests, vital signs, physical examination findings and other safety-related items.

Efficacy

The overall changes in the mean IIEF score from baseline by visit were 6.8, 0.8, 6.1, 3.4 and 5.2 at 1 month, 3 months, 6 months, 9 months and 12 months, respectively, after the injection of autologous BMSCs. These values were increased compared with baseline at all time points but were not statistically significant except at the 1-month time point (P= 0.0222) (Table 4). In some patients, the rise in IIEF score persisted until 12 months after the injection of autologous BMSCs (Figure 3).

The success rates by visit, as assessed by the SEP 2, were 30% at screening and 30%, 30%, 40%, 40% and 30% at 1 month, 3 months, 6 months, 9 months and 12 months, respectively, after the injection of autologous BMSCs. These values were not significantly different from baseline (screening) at all time points. The success rates by visit, as assessed by the SEP 3, were 0% at screening and 10%, 0%, 0%, 0% and 10% at 1 month, 3 months, 6 months, 9 months and 12 months, respectively, after the injection of autologous BMSCs. These values were not significantly different from baseline (screening) at all time

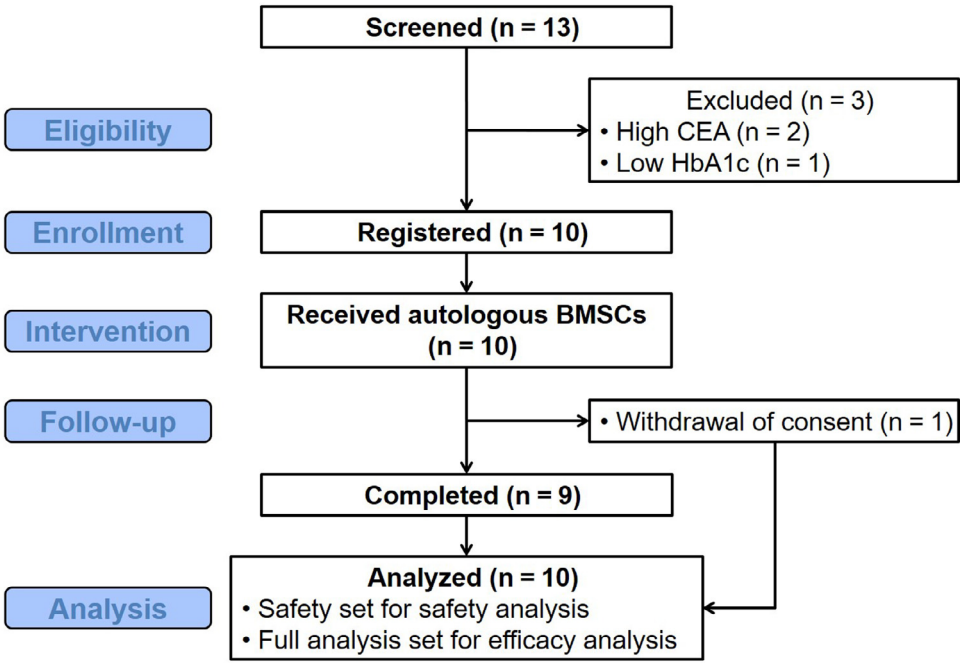

Figure 2. CONSORT diagram. CEA, carcinoembryonic antigen. (Color version of figure is available online).

Table 2
Demographic and baseline characteristics of registered patients.

	Post-prostatectomy ED (n = 5)	DM-associated ED (n = 5)	Total (N = 10)
Age, years			
Mean ± standard deviation	67.8 ± 9.1	57.0 ± 14.3	62.0 ± 13.0
Median (range)	71.5 (55.0–76.0)	53.0 (43.0–79.0)	67.0 (43.0–79.0)
Race			
Asian	5	5	10
Body mass index, kg/m^2			
Mean ± standard deviation	24.8 ± 3.1	24.7 ± 2.6	24.7 ± 2.7
Median (range)	23.4 (21.4–29.4)	23.4 (21.7–28.3)	23.4 (21.4–29.4)
Past medical history and concurrent disease			
Radical prostatectomy	5	0	5
Diabetes mellitus	0	5	5
Hypertension	0	4	4
Hyperlipidemia	0	3	3
Duration of ED, months			
Mean ± standard deviation	59.8 ± 35.7	32.8 ± 57.5	49.0 ± 44.8
Median (range)	54.0 (24.0–122.0)	5.0 (2.0–119.0)	40.5 (2.0–122.0)
Severity of ED			
Moderate	0	1	1
Severe	5	4	9

points. The percentages of patients with a yes response to the GAQ 1 by visit were 30%, 20%, 40%, 30% and 40% at 1 month, 3 months, 6 months, 9 months and 12 months, respectively, after the injection of autologous BMSCs. Among patients with a yes response to the GAQ 1, the percentages with a yes response to the GAQ 2 by visit were 100%, 100%, 75%, 100% and 50% at 1 month, 3 months, 6 months, 9 months and 12 months, respectively, after the injection of autologous BMSCs.

The overall changes in the mean PSV from baseline (screening) at 6 months and 12 months were −1.06 ± 9.81 and 3.74 ± 10.12, respectively, and both changes were not statistically significant. The overall changes in the mean EDV from baseline at 6 months and 12 months were 0.19 ± 4.67 and 0.57 ± 2.88, respectively, and both changes were not statistically significant. The overall changes in the mean RI from baseline at 6 months and 12 months were −0.02 ± 0.09 and 0.01 ± 0.07, respectively, and both changes were not statistically significant.

Discussion

PDE5Is have been the mainstay of ED treatment since the release of sildenafil in 1998 [23]. Approximately one-third of men with ED do not respond to therapy with PDE5Is, and less than one-third of men with ED due to DM or pelvic surgeries respond [4]. If a satisfactory erection is not achieved with a PDE5I, second-line therapies such as intracavernous injection, vacuum constriction device placement or intraurethral prostaglandin treatment are considered. Potential future treatments include gene therapy, penile low-intensity shock wave lithotripsy and stem cell therapy [24]. The first stem cell study for ED treatment was published in 2004 [25]. As of November 2019, there have been a total of more than 100 published basic studies and seven clinical trials assessing stem cells or their derivatives for ED treatment [8–14]. Stem cell therapy for ED is thought to have a therapeutic effect via a paracrine mechanism rather than by engraftment or transdifferentiation.

Table 3
Overall summary of TEAEs.

	Post-prostatectomy ED (n = 5)	DM-associated ED (n = 5)	Total (N = 10)
Total TEAEs	2	5	7
Patients with any TEAE (%)	1 (20)	2 (40)	3 (30)
Severity			
Mild	2	3	5
Moderate	0	2	2
Severe	0	0	0
Serious TEAEs	0	2	2
Patients with serious TEAEs (%)	0 (0)	1 (20)	1 (10)
Relationship to the clinical trial drug			
Definitely related	0	0	0
Probably related	0	0	0
Possibly related	0	0	0
Unlikely/probably not related	2	3	5
Definitely not related/none	0	2	2
Unknown/not assessable	0	0	0
Results from the clinical trial drug			
Recovery without sequelae	2	5	7
Recovery with sequelae	0	0	0
Not recovered	0	0	0
Permanent damage	0	0	0
Death	0	0	0
Unknown	0	0	0

Table 4
IIEF score and change from baseline by visit.

	Post-prostatectomy ED (n = 5)	DM-associated ED (n = 5)	Total (N = 10)
Screening			
Mean ± standard deviation	15.8 ± 5.5	20.4 ± 14.6	18.1 ± 10.7
Median (range)	16.0 (7.0–21.0)	21.0 (6.0–41.0)	18.0 (6.0–41.0)
Month 1			
Mean ± standard deviation	20.8 ± 6.3	29.0 ± 18.6	24.9 ± 13.8
Median (range)	20.0 (13.0–29.0)	25.0 (6.0–49.0)	22.5 (6.0–49.0)
Month 3			
Mean ± standard deviation	17.0 ± 12.9	20.8 ± 19.7	18.9 ± 15.8
Median (range)	12.0 (6.0–39.0)	18.0 (5.0–53.0)	14.5 (5.0–53.0)
Month 6			
Mean ± standard deviation	21.4 ± 12.2	27.0 ± 17.8	24.2 ± 14.7
Median (range)	15.0 (10.0–39.0)	22.0 (5.0–52.0)	21.0 (5.0–52.0)
Month 9			
Mean ± standard deviation	17.8 ± 14.8	25.2 ± 19.2	21.5 ± 16.6
Median (range)	10.0 (5.0–41.0)	22.0 (6.0–48.0)	16.0 (5.0–48.0)
Month 12/end of trial			
Mean ± standard deviation	19.6 ± 18.3	27.0 ± 13.8	23.3 ± 15.8
Median (range)	11.0 (9.0–52.0)	22.0 (17.0–51.0)	18.0 (9.0–52.0)
Change from baseline at 1 month			
Mean ± standard deviation	5.0 ± 7.1	8.6 ± 8.8	6.8 ± 7.8
Median (range)	8.0 (−7.0 to 10.0)	6.0 (−1.0 to 22.0)	7.0 (−7.0 to 22.0)
P value	0.1925	0.0947	0.0222
Change from baseline at 3 months			
Mean ± standard deviation	1.2 ± 12.7	0.4 ± 14.0	0.8 ± 12.6
Median (range)	−4.0 (−14.0 to 18.0)	2.0 (−22.0 to 12.0)	0.0 (−22.0 to 18.0)
P value	0.8430	0.9520	0.8452
Change from baseline at 6 months			
Mean ± standard deviation	5.6 ± 13.5	6.6 ± 6.8	6.1 ± 10.1
Median (range)	0.0 (−6.0 to 22.0)	9.0 (−2.0 to 14.0)	5.0 (−6.0 to 22.0)
P value	0.4044	0.0959	0.0874
Change from baseline at 9 months			
Mean ± standard deviation	2.0 ± 15.3	4.8 ± 6.4	3.4 ± 11.2
Median (range)	−5.0 (−11.0 to 20.0)	2.0 (−1.0 to 15.0)	1.5 (−11.0 to 20.0)
P value	0.7846	0.1698	0.3604
Change from baseline at 12 months/end of trial			
Mean ± standard deviation	3.8 ± 15.9	6.6 ± 6.1	5.2 ± 11.4
Median (range)	0.0 (−10.0 to 31.0)	10.0 (−1.0 to 12.0)	1.5 (−10.0 to 31.0)
P value	0.6210	0.0730	0.1844

The mechanism of action is thought to be mainly due to prevention of corporeal smooth muscle deterioration and collagen deposition in the corpus cavernosum and possibly the regeneration of some cavernous nerves [17,18]. This phase 1 clinical trial was planned to evaluate the safety and potential efficacy of intracavernously injected autologous BMSC therapy in patients with ED unlikely to recover with PDE5I treatment.

The primary outcome of the clinical trial was to evaluate the safety of autologous BMSC treatment in patients with ED. Three out of 10 patients experienced seven TEAEs, and one patient with DM-

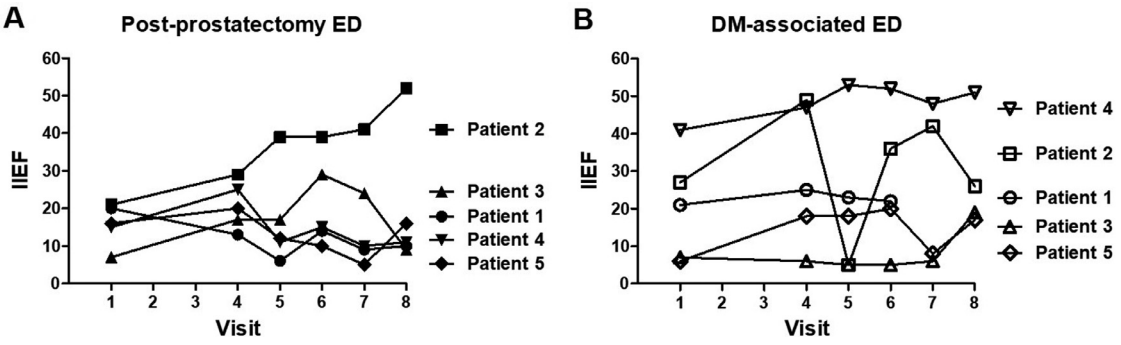

Figure 3. IIEF score of individual patients from baseline by visit. (A) Post-prostatectomy ED. (B) DM-associated ED.

associated ED experienced two serious TEAEs that were classified as hyperglycemia of metabolism and nutrition disorders according to the MedDRA system organ class and preferred term. However, all TEAEs were considered not to be related to autologous BMSC therapy, and no TEAE resulted in death or dropout during the clinical trial. Moreover, no abnormal CS was observed for laboratory tests, vital signs, physical examination findings and other safety-related items after the injection of autologous BMSCs. Previous clinical trials using various cultured or uncultured regenerative cells for ED treatment described no serious TEAEs during or after treatment [8–14]. Importantly, none of the patients showed changes in tumor marker tests, including PSA levels, to abnormal CS. In a French phase 1/2 clinical trial of autologous bone marrow mononuclear cell injection in 12 patients with post-prostatectomy ED, no evidence of prostate cancer recurrence was observed during a mean follow-up time of 5 years after the injection [26].

The secondary outcome of the clinical trial was to evaluate the potential efficacy of autologous BMSC treatment in patients with ED by examining improvement in erectile function and patient satisfaction and by observing hemodynamic changes in the penis using PDS. The IIEF score was evaluated as an indicator of erectile function. In the present study, the IIEF score was significantly increased compared with the baseline score at only the 1-month time point. In a French trial, significant improvements in the erectile function and intercourse satisfaction domains of the IIEF score were observed at 6 months after the injection [10,26]. In a Jordanian phase 1 clinical trial of two autologous BMSC injections in four patients with DM-associated ED, a significant improvement in the IIEF score was observed until 12 months after the injection [13]. In a private practice urology study conducted in the US using placental matrix-derived MSCs in eight patients with organic ED, the change in the IIEF score was not statistically significant until 6 months after the injection [12].

The SEP 2 and 3 and GAQ 1 and 2 were evaluated as indicators of patient satisfaction. The successful vaginal penetration rate was maintained at 30–40% until 12 months after the injection. The successful intercourse rate was 10% at 1 month and 12 months after the injection. In addition, 20–40% of patients answered that autologous BMSC treatment improved their erectile function, and 50–100% answered that this improved their ability to engage in sexual activity.

Both the PSV and EDV were evaluated as indicators of hemodynamic changes in the penis. In the present study, both PSV and EDV changes were not statistically significant compared with baseline at 6 months and 12 months. In a French trial, the PSV increased significantly after the injection, and clinical benefits were associated with improvement in PSV [10]. In a private practice urology study conducted in the US, the PSV increased by a statistically significant extent at 3 months and 6 months after the injection [12]. In a Greek phase 1 clinical trial of injection of autologous adipose-derived MSCs with platelet lysate in five patients with organic ED, the PSV tended to improve until 3 months after the injection [14].

The main limitation of the present study was the small sample size. In addition, a relatively small number of BMSCs were administered only once. As mentioned previously, the dose and number of BMSCs were chosen based on the results of a previous pre-clinical study [18]. However, the authors cannot rule out the possibility of better effects from a larger number of stem cells or multiple injections [13,27]. Finally, the present study was unblinded and did not include a control group. Therefore, the possibility that the efficacy of BMSC therapy was a placebo effect cannot be excluded. Naturally, a randomized controlled trial would be needed to clarify the benefits of this treatment compared with a control group. The authors have been performing a phase 2 clinical trial to evaluate the efficacy of autologous BMSC therapy for post-prostatectomy ED based on the current results and those of a previous report on efficacy outcomes [28].

Conclusions

Since there were no TEAEs related to autologous BMSC therapy in this phase 1 clinical trial, autologous BMSC therapy is considered a safe treatment for patients with ED. The potential efficacy of autologous BMSC treatment in patients with ED needs to be confirmed by a randomized, placebo-controlled clinical trial.

Declaration of Competing Interest

HCS is an employee of Pharmicell Co, Ltd, and YMK was an employee of Pharmicell Co, Ltd, during the clinical trial.

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Author Contributions

Conception and design of the study: DY, HCS, YMK, TYA and CSK. Acquisition of data: MJJ and GS. Analysis and interpretation of data: NS. Drafting or revising the manuscript: DY and HCS. All authors have approved the final article.

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