Efficacy and Safety of Stempeucel in Osteoarthritis of the Knee

A Phase 3 Randomized, Double-Blind, Multicenter, Placebo-Controlled Study

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Background: Osteoarthritis is a chronic, progressive, and degenerative condition with limited therapy options. Recently, biologic therapies have been an evolving option for the management of osteoarthritis.

Purpose: To assess whether allogenic mesenchymal stromal cells (MSCs) have the potential to improve functional parameters and induce cartilage regeneration in patients with osteoarthritis.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A total of 146 patients with grade 2 and 3 osteoarthritis were randomized to either an MSC group or placebo group with a ratio of 1:1. There were 73 patients per group who received either a single intra-articular injection of bone marrow–derived MSCs (BMMSCs; 25 million cells) or placebo, followed by 20 mg per 2 mL of hyaluronic acid under ultrasound guidance. The primary endpoint was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total score. The secondary endpoints were WOMAC subscores for pain, stiffness, and physical function; the visual analog scale score for pain; and magnetic resonance imaging findings using T2 mapping and cartilage volume.

Results: Overall, 65 patients from the BMMSC group and 68 patients from the placebo group completed 12-month follow-up. The BMMSC group showed significant improvements in the WOMAC total score compared with the placebo group at 6 and 12 months (percentage change: −23.64% [95% CI, −32.88 to −14.40] at 6 months and −45.60% [95% CI, −55.97 to −35.23] at 12 months $P < .001$; percentage change, −44.3%). BMMSCs significantly improved WOMAC pain, stiffness, and physical function subscores as well as visual analog scale scores at 6 and 12 months ($P < .001$). T2 mapping showed that there was no worsening of deep cartilage in the medial femorotibial compartment of the knee in the BMMSC group at 12-month follow-up, whereas in the placebo group, there was significant and gradual worsening of cartilage ($P < .001$). Cartilage volume did not change significantly in the BMMSC group. There were 5 adverse events that were possibly/probably related to the study drug and consisted of injection-site swelling and pain, which improved within a few days.

Conclusion: In this small randomized trial, BMMSCs proved to be safe and effective for the treatment of grade 2 and 3 osteoarthritis. The intervention was simple and easy to administer, provided sustained relief of pain and stiffness, improved physical function, and prevented worsening of cartilage quality for ≥12 months.

Registration: CTRI/2018/09/015785 (National Institutes of Health and Clinical Trials Registry–India).

Keywords: osteoarthritis; mesenchymal stromal cells; WOMAC; T2 mapping

Osteoarthritis is a chronic, progressive condition that is degenerative in nature and characterized by a gradual loss of cartilaginous tissue, leading to stiffness, pain, and impaired movement of the affected joint. The disease most commonly affects the joints in the hips, knees, spine, feet, and hands. Its modifiable and nonmodifiable risk

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factors include obesity, genetic predisposition, lack of exercise, trauma, sex, and age.[37] It is now evident that osteoarthritis is not only caused by wear and tear but also by the involvement of various proinflammatory cytokines and mediators.[5]

According to the World Health Organization, 18.0% of women and 9.6% of men >60 years of age have symptomatic osteoarthritis worldwide; 80% of them have limitations in movement, and in 25%, their quality of life is majorly affected.[30] In Indian populations, osteoarthritis of the knee is most prevalent, followed by osteoarthritis of the hand. In India, around 23.46 million people had osteoarthritis in 1990, which increased to 62.35 million in 2019.[38]

Current management approaches for osteoarthritis include primary prevention (weight loss, averting joint injuries) and clinical treatment, which focuses on improving function, pain, and quality of life while avoiding therapeutic toxicity.[20] Surgical interventions include osteotomy as well as unicondylar and total knee replacement.[36] From a societal viewpoint, osteoarthritis is costly, with high direct costs in the form of increased utilization of hospital and medical services and high indirect costs through lost productivity of patients and their caregivers.[45]

Mesenchymal stromal cells (MSCs), which have the potential for cartilage regeneration in osteoarthritis, have been presented as an alternative source for cell-based therapy to chondrocytes.[27] The regenerative potential of MSCs is of interest in osteoarthritis, as very few treatment modalities have been shown to reverse or stop the loss of cartilage.[40] Immunomodulatory and anti-inflammatory properties of MSCs may be of value in osteoarthritic joints, regardless of their regenerative capacity.[41] In our published phase 2 study, we had shown that the intra-articular administration of the lowest dose of 25 million cultured, pooled, and allogenic MSCs (Stempeucel; Stempeutics) was safe and demonstrated a positive trend of improvement in pain, stiffness, and physical function of the affected joint.[11] The purpose of this study was to conduct a randomized, double-blind, placebo-controlled phase 3 study using allogenic, cultured, and pooled bone marrow–derived MSCs (BMMSCs) for the management of symptomatic osteoarthritis of the knee. Our hypothesis was that BMMSCs would result in improved function and decreased pain compared with placebo; however, a null hypothesis was also proposed that BMMSCs would have no improvement in efficacy parameters compared with placebo.

METHODS

Preparation and Characterization of BMMSCs

The investigational medicinal product (IMP) was BMMSCs, which are bone marrow–derived, ex vivo–expanded, pooled, allogenic human MSCs that have been characterized in our previous publications.[10-12,25] Cells from 3 healthy donors meeting the inclusion criteria (see Appendix 1, available in the online version of this article) were used to produce the BMMSCs in an approved good manufacturing practice facility. The final pooled product was released at passage 5 (US patent No. 8956862; February 17, 2015). A total of 25 million expanded BMMSCs were cryopreserved in 1 mL of CryoStor CS5 (Sigma-Aldrich) in blinded 5-mL Crystal Zenith vials (West Pharmaceutical Services). Placebo was contained in 1 mL of CryoStor CS5 in similar blinded cryovials. The in-house IMP specifications are given in Table 1. Details on cell manufacturing and release criteria are provided in Appendices 1 and 2 (available online), respectively.

Viability and Viable Cell Count

The vial containing 25 million BMMSCs was thawed in a 37°C water bath and analyzed for viability and viable cell count in a Vi-Cell XR cell viability analyzer (Beckman Coulter). Viable and nonviable cells appeared brighter and darker, respectively, after trypan blue staining and were automatically recognized by the instrument using viable cell spot area and viable cell spot brightness settings. The viability percentage and the total number of viable cells were determined from the average of 50 images.

Potency Assay Using Thrombospondin-2 (TSP-2)

The secretion of thrombospondin-2 (TSP-2) by BMMSCs was determined using a potency assay to measure their in vitro chondrogenic potential. For analysis of the secretome, 1 million MSCs at passage 5 were plated in a 75-cm^2 flask in knockout Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 2 mM of L-glutamine, 1X penicillin-streptomycin (Invitrogen), and 2 ng/mL of basic fibroblast growth factor (Invitrogen) and cultured for 72 hours. The spent medium was collected and used for analysis with the TSP-2 ELISA Kit (R&D

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TABLE 1
Investigational Medicinal Product Specifications

No.	Test	Specification
1	Description	Cells are fibroblastic and spindle-shaped when actively growing; they are intact and round in shape after trypsinization
2	Viable cell count	≥20 million cells
3	Viability	≥80%
4	Bacterial endotoxins	<0.125 EU/mL
5	Mycoplasma by polymerase chain reaction–enzyme-linked immunosorbent assay	Not detected
6	Sterility	No microbial growth
7	Purity	CD105 ≥90%, CD73 ≥90%, CD34 ≤10%, CD45 ≤10%
8	Differentiation assay for adipocytes, chondrocytes, and osteocytes	Confirmation of differentiation
9	Infectious disease markers by polymerase chain reaction (human immunodeficiency virus, hepatitis C virus, hepatitis B virus, Epstein-Barr virus, parvovirus B19, cytomegalovirus)	Negative
10	Karyotype	Normal (46,XY)
11	Potency assay for thrombospondin-2 secretion levels	≥10 ng/mL/million cells

Systems) by following the manufacturer's instructions. The level of TSP-2 was estimated at an absorbance of 450 nm using a VersaMax microplate reader (Molecular Devices).

Regulations in India for Cell Therapy Products

In India, per the New Drugs and Clinical Trials Rules of 2019, issued by the Central Drugs Standard Control Organisation (CDSCO), a cell therapy product is considered to be a drug and is called a cell- or stem cell–derived product. The National Guidelines for Stem Cell Research issued by the Indian Council of Medical Research define the minimal manipulation, substantial or more than minimal manipulation, and major manipulation of cells.[15] They state that clinical trials using cells that have undergone more than minimal manipulation, which includes the culture expansion of cells harvested from bone marrow, adipose tissue, or any other source, can only be conducted after obtaining approval from the CDSCO.

Study Design and Enrollment Criteria

This study was registered prospectively with the National Institutes of Health and Clinical Trials Registry–India (No. CTRI/2018/09/015785) and planned as a randomized, double-blind, multicenter, placebo-controlled phase 3 study. The study assessed the efficacy and safety of a single intra-articular injection of 25 million cells compared with a single dose of placebo in patients with knee osteoarthritis. The study was conducted in accordance with good clinical practice guidelines issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH/135/95; July 2002) and principles outlined in the Declaration of Helsinki (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013). Approval was obtained from the CDSCO and the institutional ethics committees of 15 participating hospitals. An independent data safety monitoring board was established comprising drug safety physicians and experts in therapeutic disciplines to monitor safety data at predefined intervals during the study. The study was started in January 2019, and 1-year follow-up of the patients was completed in April 2021. Written informed consent was obtained from all participants before screening.

Patients were prospectively stratified and randomized by block randomization, with a block size of 4 patients each for grade 2 and 3 osteoarthritis. The unblinded biostatistician generated the randomization protocol using PROC PLAN in SAS (Version 9.2 or later; SAS Institute) and shared it with an independent, unblinded representative of the IMP management team. The investigators (S.M., J.J.C., V.G., A.K.S., S.K.T., K.T., V.P., P.K.S., S.S., S.Ba, N.S., S.U.K., P.S.P.), patients, study-site personnel, central laboratory personnel, central radiologist, sponsors (P.K.G., J.A., S.K., S.Bh, S.P., U.K., V.S., A.S.), and clinical research personnel remained blinded except 1 person each from the IMP management team and biostatistics team. The eligibility criteria of the patients enrolled in the trial are shown in Table 2.

Intervention

Of the 426 patients who provided informed consent, 146 patients met the eligibility criteria and were randomized. A total of 73 patients each from the BMMSC and placebo groups received a dose of the study treatment (CONSORT [Consolidated Standards of Reporting Trials] flowchart in Figure 1). Patients randomized to the BMMSC arm received a single intra-articular injection of 25 million BMMSCs suspended in 2 mL (20 mg) (1 mL of CryoStor CS5 + 1 mL of Plasma-Lyte A [Baxter]) of medium, followed by an injection of 2 mL of hyaluronic acid. Patients in the placebo arm received an intra-articular injection of 2 mL of placebo (1 mL of CryoStor CS5 + 1 mL of Plasma-Lyte A), followed by 2 mL of hyaluronic acid. All intra-articular injections were administered under ultrasound guidance. Both the cells and placebo were supplied in blinded cryovials that were indistinguishable from each other, and the injections were administered using a 5-mL blinded syringe. Medication before the administration of the IMP as well as the

TABLE 2
Participant Eligibility Criteria

Inclusion Criteria
1. Male and female patients aged 40-65 y (both inclusive)
2. Body mass index <30
3. History of primary osteoarthritis of the knee characterized by pain, requiring the intake of analgesics
4. Radiograph of the knee joint showing evidence of grade 2 to 3 osteoarthritis based on the Kellgren-Lawrence classification
5. Self-reported difficulty in ≥1 of the following activities attributed to knee pain: lifting and carrying groceries, walking 400 m, getting in and out of a chair, getting up from a squatting or cross-leg position, or going up and down the stairs
6. Use of analgesic medication for osteoarthritis for 6 wk based on the investigator's judgment
7. Willingness to refrain from any other stem cell treatment for 2 y during the study period
8. Female patients of childbearing age who were willing to use accepted methods of contraception during the course of the study
9. Willingness to provide written informed consent including audiovisual consent
Exclusion Criteria
1. Radiograph (evaluated by the central radiologist) showing any of the following:
a. Grade 0, 1, and 4 osteoarthritis based on the Kellgren-Lawrence classification
b. Subchondral sclerosis (involving both the medial and the lateral femorotibial compartments of the knee joint)
2. Magnetic resonance imaging scan of the knee showing any of the following:
a. Anterior cruciate ligament/posterior cruciate ligament tears (complete tears were excluded)
b. Grade 3 meniscal tears, defined as increased signal intensity on proton density–weighted sequences extending up to either articular surface; this also included root tears but excluded ramp tears (grade 3 complete root tears only were excluded)
c. Exclusive patellofemoral arthritis
d. Grade 0, 1, and 4 osteoarthritis per the proposed grading system
3. Prior or ongoing medical conditions (eg, concomitant illness, psychiatric condition), alcoholism, smoking, tobacco chewing or drug abuse, medical history results, physical examination findings, electrocardiography findings, or laboratory abnormalities that, in the investigator's opinion, could adversely affect the safety of the patient, make it unlikely that the course of treatment or follow-up would be completed, or impair analysis of the study results
4. History of surgery or major trauma to the examined joint
5. Arthroscopic surgery on the examined joint in the previous 12 mo
6. Signs of active joint inflammation including redness, warmth, and/or large, bulging effusion with a loss of the normal contour of the joint at the screening visit or on the baseline examination
7. Acute exacerbation of the examined joint in the past 6 wk
8. Use of intra-articular steroids or hyaluronan within the past 3 mo
9. Any stem cell treatment in the past by any route of administration
10. Infection in or around the examined knee
11. Awaiting replacement of the knee or hip joint
12. Other conditions that cause pain in the knee joint
13. Gross deformity (varus deformity on radiography of the knee or flexion deformity >10° using a goniometer) of the knee joint based on the principal investigator's judgment
14. Significantly incapacitated or disabled patient categorized as American College of Rheumatology class IV of functional status (largely or wholly incapacitated) or inability to walk without assistive devices
15. Any secondary causes of arthritis (eg, rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, psoriasis, rheumatic or inflammatory disease)
16. Body mass index ≥30
17. Hepatitis B surface antigen, hepatitis C antibody, human immunodeficiency virus 1 and 2 antibodies, positive *Treponema pallidum* hemagglutination assay test finding, or cytomegalovirus (IgM) antibody
18. History of bleeding disorders
19. Known hypersensitivity to hyaluronan products, animal sera, or constituents of the investigational medicinal product
20. Pregnancy, breastfeeding, planned pregnancy during the study period, or women of childbearing potential not using adequate contraception

injection protocol were similar to those published earlier.[11] Patients were discharged at 24 hours after an inspection of the target knee joint and a general physical examination and vital signs.

Outcomes

The primary endpoint of the study was to assess the change in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total score in the BMMSC arm from baseline to 1 year compared with the placebo arm. Scores for each WOMAC subscale ranged from 0 to 500 for pain, 0 to 200 for stiffness, 0 to 1700 for physical function, and the WOMAC total score ranged from 0 to 2400. A visual analog scale (VAS) was also administered, which was a measure of pain intensity, with scores ranging from 0 to 100. The minimal clinically important difference (MCID) was used to analyze patient-reported outcome

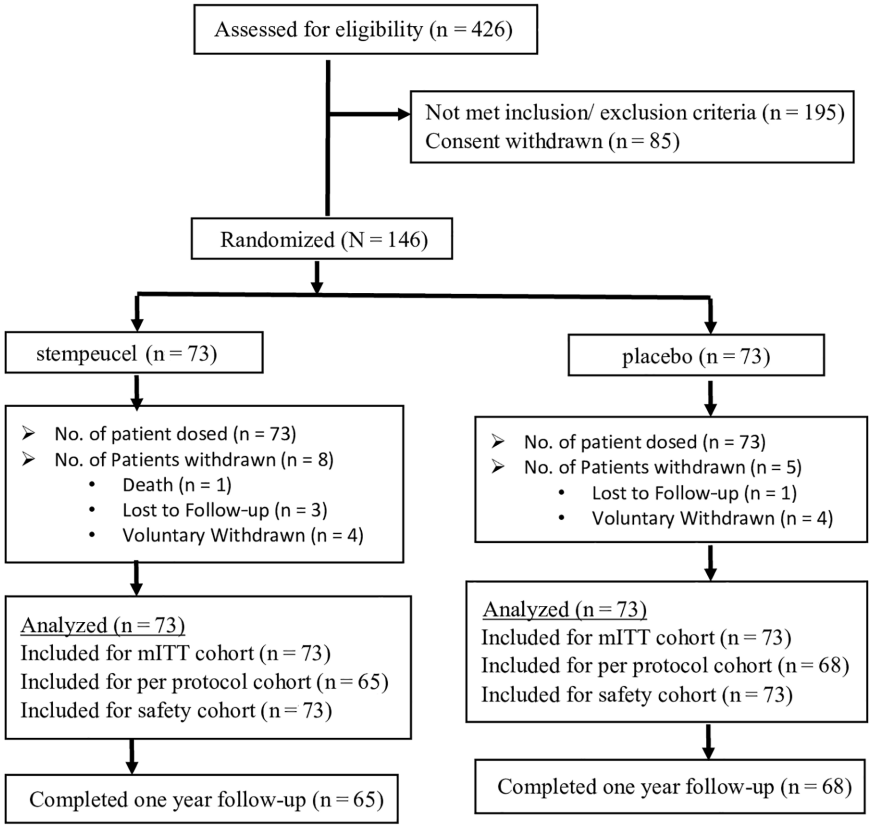

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart showing the number of patients screened, randomized, followed up, and analyzed. mITT, modified intention to treat.

measures (PROMs) such as the WOMAC total and VAS. A ≥20% difference in scores between the study groups was considered to have a substantial clinical benefit.[1,35]

The secondary efficacy endpoints included the WOMAC subscores of pain, stiffness, and physical function; the VAS score; and magnetic resonance imaging (MRI) findings of cartilage quality using T2 mapping and cartilage volume in the BMMSC arm compared with the placebo arm at the 1-year time point. MRI was conducted at baseline, 6 months, and 1 year using a 3-T machine of ≥8 channels and with an extremity coil/flex coil using CartiGram T2 sequence software (GE Healthcare). Sagittal T2-weighted images of each patient were loaded and color-coded with the IntelliSpace Portal (Philips), which divided each sagittal image into 6 predefined subcompartments and then further subdivided each subcompartment into 3 layers (superficial, deep, and intermediate) for detailed cartilage analysis. T2 relaxation times of cartilage were calculated for each subdivision (with T2 being higher in damaged cartilage). Cartilage volume was calculated manually using 2 separate 5-mm 3-dimensional sagittal images in maximum intensity projection. Knee MRI assessments were performed by a single radiologist blinded for all the parameters at all time points.

The secondary safety endpoints included all adverse events (AEs), treatment-emergent AEs (TEAEs), and laboratory parameters. Exploratory endpoints included the presence of the biomarker C-terminal cross-linked telopeptide of type II collagen (CTX-II; indicative of disease progression) in urine and the anti-inflammatory marker interleukin 10 (IL-10) in serum.

Follow-up

Clinical and laboratory outcomes were evaluated at 1 week and 1, 3, 6, and 12 months after the administration of the IMP. The clinical data were unblinded after 12 months of follow-up, and the patients were followed up for efficacy and safety for 24 months after the injection.

Sample Size Calculation

The sample size was calculated using nQuery software (Version 6; Statsols). The input was determined based on the change in the WOMAC total score from baseline to 6 months for the MSC and placebo arms from the completed phase 2 study.[13] To establish the superiority of a dose of 25 million BMMSCs compared with placebo with 90% power and alpha of .05, a 2-sided test was applied, and the study required 51 evaluable patients per group. To account for a dropout rate of 30%, the study required 73 evaluable patients per group for a total of 146 patients.

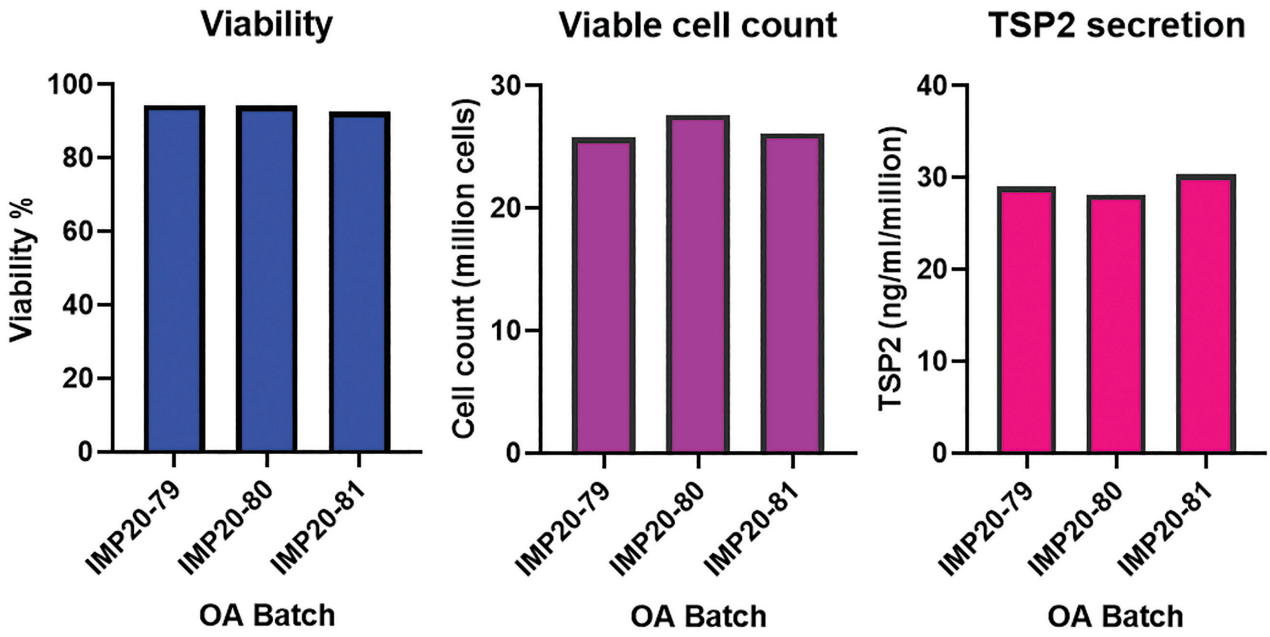

Figure 2. Potency assay of bone marrow–derived mesenchymal stromal cells showing viability, viable cell count, and thrombospondin-2 (TSP-2) secretion. The viability of all the batches was >90%, with a mean cell yield of 26.5 million and TSP-2 secretion level of 29.20 ng/mL/million cells. IMP, investigational medicinal product; OA, osteoarthritis.

Statistical Analysis

The SAS package (Version 9.4; SAS Institute) was used for statistical analysis. There were 3 cohorts considered for analysis: the modified intention-to-treat (mITT), per-protocol (PP), and safety cohorts. The mITT cohort was used for analysis of the primary efficacy endpoint, and the PP cohort was also used to analyze the primary endpoint. Analysis of the secondary efficacy endpoints was performed using the mITT cohort. The mITT cohort included all randomized patients who received the study medication and had a baseline measurement and $\geq$1 posttreatment measurement, whereas the PP cohort included all randomized patients who completed both the baseline visit and the end-of-treatment visit and had no major protocol violations/deviations. The normality of data was examined with the Shapiro-Wilk test. Based on the normality test, an independent t test/paired t test or the nonparametric Mann-Whitney test/Wilcoxon signed-rank test was performed for comparisons of the change from baseline values between the study groups using the mITT and PP cohorts at a 5% level of significance. If $P < .05$, then the null hypothesis would be rejected. AEs were summarized descriptively by the total number of AEs and compared between the 2 study groups. AEs and TEAEs were presented as the number and proportion of patients who experienced AEs by treatment group, classified by the Medical Dictionary for Regulatory Activities "System Organ Class" and "Preferred Term." All AEs were categorized in terms of severity (mild, moderate, and severe), action taken, relatedness, expectedness, and outcome. Certain secondary efficacy endpoints and exploratory biomarkers were analyzed using a generalized estimating equation (GEE) model with longitudinal analysis.[13,43]

RESULTS

Viability, Viable Cell Count, and TSP-2 Secretion

Analysis of cell viability and count is presented in Figure 2. On analysis, we found that the viability of the different cell batches analyzed was >90%, and the mean viable cell count was 26.5 $\pm$ 0.9 million cells in a vial. As TSP-2 plays a major role in MSC-mediated cartilage regeneration, we analyzed the potency of MSCs to secrete TSP-2 in culture from 3 different BMMSC batches. All batches showed uniform levels of TSP-2 secretion, and the values of the 3 batches were 29.03, 28.19, and 30.39 ng/mL/million cells.

Baseline Characteristics of Patients

The baseline characteristics of the enrolled patients are presented in Table 3. Both the BMMSC and the placebo groups were comparable and balanced at baseline in all the parameters. High scores for the VAS and WOMAC suggest that the patients enrolled in the study had severe pain, and the baseline data were balanced in the efficacy parameters in both groups. There were no major protocol deviations observed during the study.

Safety Profile

A total of 79 TEAEs were reported in 44 patients (Table 4). The majority of the TEAEs in both arms of the study were mild and moderate in severity. One TEAE in 1 patient (1.4%) in the BMMSC arm had a fatal outcome (coronavirus infection). Additionally, 5 TEAEs (n = 4 [5.5%] in

TABLE 3
Patient Characteristics at Baseline[a]

	BMMSC (n = 73)	Placebo (n = 73)
Age, y	51.6 ± 6.77	53.6 ± 6.78
Height, cm	159.0 ± 6.89	157.8 ± 7.74
Weight, kg	67.1 ± 8.41	65.4 ± 9.05
Body mass index	26.5 ± 2.58	26.2 ± 2.99
Sex, male:female, n	26:47	22:51
Kellgren-Lawrence grade of osteoarthritis, n		
Grade 2	36	38
Grade 3	37	35
WOMAC total score	1412.0 ± 336.59	1361.4 ± 305.18
WOMAC pain subscore	295.3 ± 76.03	282.0 ± 67.70
WOMAC stiffness subscore	114.6 ± 36.76	109.4 ± 35.17
WOMAC physical function subscore	1002.7 ± 236.47	969.9 ± 223.40
VAS score	66.1 ± 13.96	65.0 ± 13.09

[a]Data are shown as mean ± SD unless otherwise indicated. BMMSC, bone marrow–derived mesenchymal stromal cell; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

TABLE 4
Treatment-Emergent Adverse Events[a]

	BMMSC (n = 73)	Placebo (n = 73)
Total	49 (24)	30 (20)
Ear and labyrinth disorders	1 (1)	0 (0)
Gastrointestinal disorders	3 (2)	4 (3)
General disorders and administration site conditions	10 (7)	4 (3)
Infections and infestations	9 (6)	4 (4)
Injury, poisoning, and procedural complications	2 (2)	1 (1)
Investigation related	1 (1)	4 (4)
Metabolism and nutrition disorders	4 (4)	7 (6)
Musculoskeletal and connective tissue disorders	13 (8)	1 (1)
Nervous system disorders	0 (0)	3 (3)
Respiratory, thoracic, and mediastinal disorders	3 (3)	0 (0)
Skin and subcutaneous tissue disorders	2 (2)	1 (1)
Vascular disorders	1 (1)	1 (1)

[a]Data are shown as number of events (number of patients). BMMSC, bone marrow–derived mesenchymal stromal cell. Some patients had more than one adverse event.

BMMSC group and n = 1 [1.4%] in placebo group) were considered to be possibly/probably related to the study drug. These included injection site joint swelling, injection site pain, and injection site joint pain. All events of joint pain and swelling recovered completely within a few days of symptomatic treatment. There were 10 serious AEs (SAEs) in 8 patients reported during the study. Overall, 6 SAEs in 4 patients (5.5%) recorded in the BMMSC group consisted of intestinal obstruction (2 events), coronavirus infection (2 events), gastroenteritis, and severe fever with thrombocytopenia; 4 SAEs in 4 patients (5.5%) recorded in the placebo group comprised coronavirus infection (2 events), transient ischemic attack, and hypertension. All the events were assessed as unrelated to the study drug. One patient in the BMMSC arm had 3 SAEs: gastroenteritis (1 event) and intestinal obstruction (2 events). With regard to the SAE of intestinal obstruction, the patient during the first admission underwent initial laparoscopy, followed by laparotomy and a surgical intervention, and during her second admission was managed nonoperatively. The patient improved and was discharged in a hemodynamically stable state. Also, 2 patients each in the BMMSC and placebo arms developed a coronavirus infection. There were 3 patients who recovered from the infection; however, 1 patient in the BMMSC arm developed complications of the coronavirus infection and died because of septicemia with a septic acute kidney injury and bilateral pneumonitis. The cause of the SAE was assessed by the investigator, ethics committee, and independent data safety monitoring board as not related to BMMSCs. Another patient developed fever with thrombocytopenia (decreased to 43,000/mm^3), which was initially diagnosed as dengue fever, but the NS1 antigen test result was negative. The patient was managed nonoperatively, and the patient improved and was discharged under stable conditions. The SAE was determined to be unrelated to the study drug.

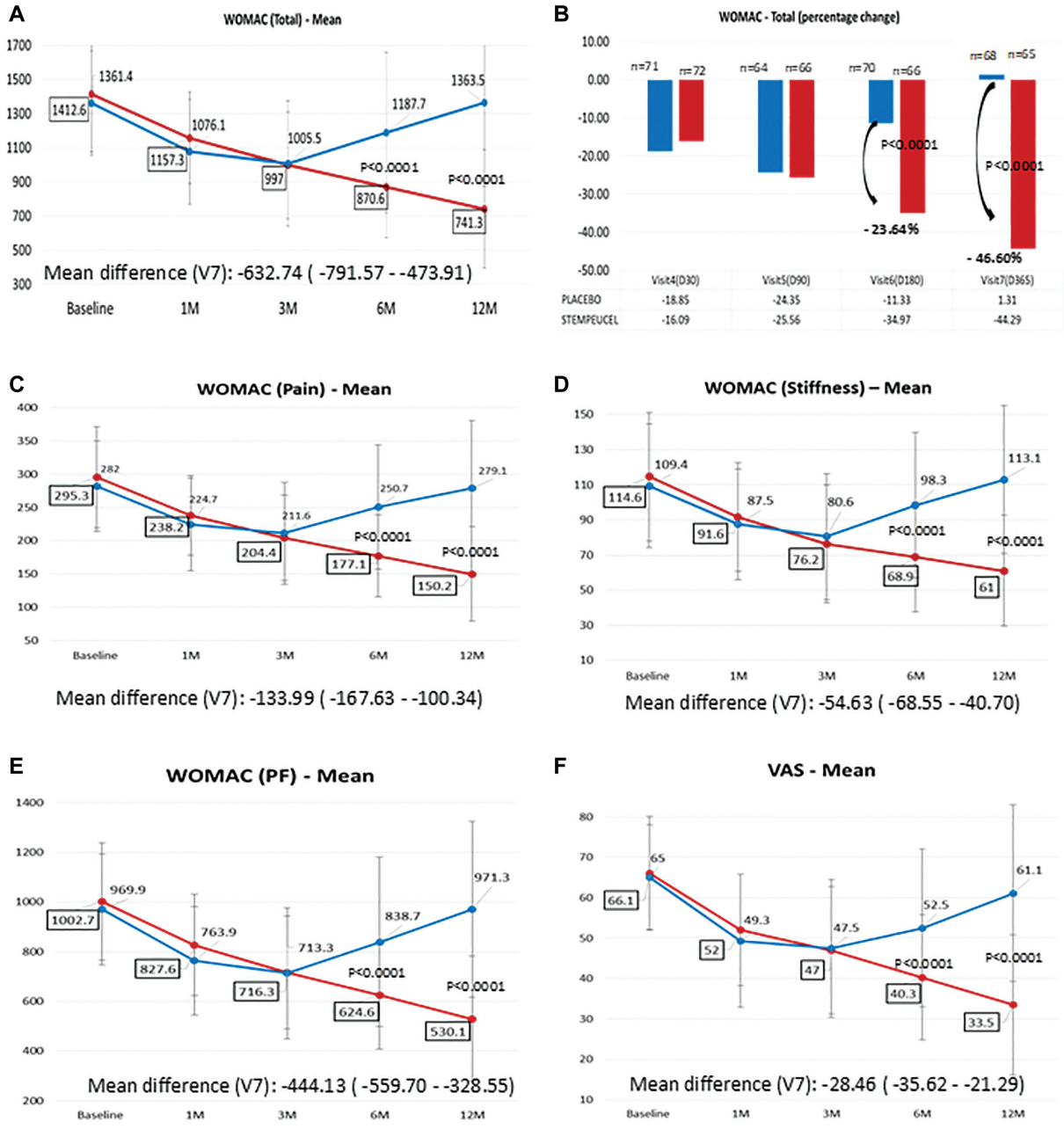

Figure 3. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total score and subscores. (A) Mean values of the WOMAC total score over time. (B) Percentage change in the WOMAC total score over time. (C) Mean values of the WOMAC pain subscore over time. (D) Mean values of the WOMAC stiffness subscore over time. (E) Mean values of the WOMAC physical function (PF) subscore over time. (F) Mean values of the visual analog scale (VAS) score over time. Red line indicates Stempeucel arm and the blue line indicates placebo arm.

All hematological and biochemical parameters including electrocardiography results did not reveal any significant abnormalities. There was no difference in the incidence of AEs between the BMMSC and the placebo arms. A total of 4 patients underwent total knee replacement at 1-year follow-up: 3 patients in the placebo arm and 1 patient in the BMMSC arm.

Clinical Outcomes

The WOMAC total score decreased in both the BMMSC and placebo arms at 3 months of follow-up (997.0 ± 312.56 vs 1005.5 ± 367.21, respectively; $P = .3905$). Thereafter, the mean score in the BMMSC arm was lower compared with the placebo arm at 6 months (870.6 ± 297.87

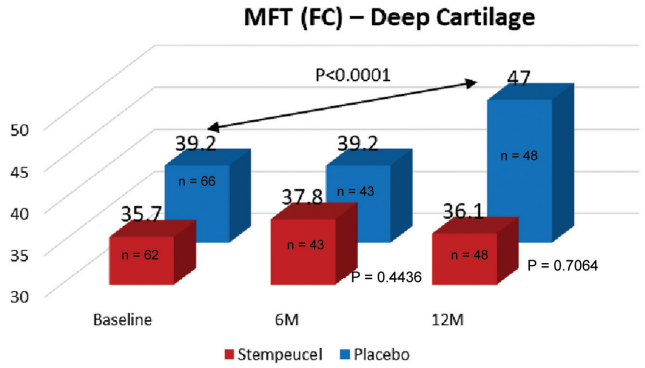

Figure 4. T2 mapping of change from baseline scores in ms in deep cartilage of the medial femorotibial (MFT [femoral condylar]) compartment.

vs 1187.7 ± 469.69, respectively; $P < .001$) and 12 months (741.3 ± 346.13 vs 1363.5 ± 488.62, respectively; $P < .001$) (Figure 3A). The mean percentage difference between the groups showed a statistically significant improvement ($P < .001$) in the BMMSC arm both at 6 months (−23.64 [95% CI, −32.88 to −14.40]) and 12 months (−46.60 [95% CI, −55.97 to −35.23]) (Figure 3B). Also, 89.2% of patients in the BMMSC group showed an improvement of ≥20% compared with 26.5% in the placebo group.

WOMAC subscores showed a similar trend; the mean difference in the WOMAC pain subscore at 6 months was −83.49 (95% CI, −113.61 to −53.38) and at 12 months was −133.99 (95% CI, −167.63 to −100.34) in the BMMSC arm compared with the placebo arm (both $P < .001$) (Figure 3C). A mean percentage reduction was observed in the BMMSC arm of −26.91 (95% CI, −36.29 to −17.53) at 6 months and −46.34 (95% CI, −56.94 to −35.73) at 12 months, showing a statistically significant improvement ($P < .001$).

The mean difference in the WOMAC stiffness subscore at 6 months was −33.44 (95% CI, −46.61 to −20.27) and at 12 months was −54.63 (95% CI, −68.55 to −40.70) in the BMMSC arm compared with the placebo arm (both $P < .001$) (Figure 3D). A mean percentage reduction was observed in the BMMSC arm of −31.90 (95% CI, −44.72 to −19.08) at 6 months and −55.12 (95% CI, −79.35 to −30.89) at 12 months, showing a statistically significant improvement ($P < .001$).

The mean difference in the WOMAC physical function subscore at 6 months was −232.98 (95% CI, −337.03 to −128.92) and at 12 months was −444.13 (95% CI, −559.70 to −328.55) in the BMMSC arm compared with the placebo arm (both $P < .001$) (Figure 3E). A mean percentage reduction was observed in the BMMSC arm of −22.60 (95% CI, −32.07 to −13.12) at 6 months and −45.63 (95% CI, −56.27 to −34.98) at 12 months (both $P < .001$).

The VAS score decreased in both study groups at 3-month follow-up; thereafter, the decrease was seen only in the BMMSC arm. The mean score at 12-month follow-up was 33.5 ± 17.34 and 61.1 ± 21.85 in the BMMSC and placebo arms, respectively, and a mean difference of −28.46 (95% CI, −35.62 to −21.29) was observed with statistical significance for the BMMSC arm ($P < .001$) (Figure 3F). A mean percentage reduction was observed in the BMMSC arm of −17.62 (95% CI, −26.20 to −9.04) at 6 months and −41.58 (95% CI, −51.86 to −31.29) at 12 months (both $P < .001$). Also, 89.2% of patients in the BMMSC group showed an improvement of ≥20% compared with 29.4% in the placebo group.

Structural Outcomes on MRI

T2 mapping of deep cartilage of the medial femorotibial compartment showed the relaxation time within normal limits (<40 ms) in the BMMSC arm at baseline (35.7 ± 7.68 ms), 6 months (37.8 ± 8.72 ms), and 12 months (36.1 ± 7.79 ms). However, in the placebo arm, the relaxation time gradually increased from baseline (39.2 ± 8.04 ms) to 6 months (39.2 ± 8.07 ms) and then 12 months (47.0 ± 75.20 ms), which was statistically significant ($P < .001$) (Figure 4). However, the difference between the 2 groups was not significant at 6- and 12-month follow-up. The lateral femorotibial compartment did not show any changes in both the groups. Representative images of T2 mapping are shown in Figure 5.

Cartilage volume was evaluated by the GEE method and showed an increase in an average volume of 34.07 units as compared to placebo arm irrespective of time, which was statistically not significant.

Biomarker Analysis

Interleukin 10. In the BMMSC arm, values increased significantly compared with baseline at 1 month (0.376 ± 2.0721 pg/mL; $P = .0313$) but not at 3 months (0.323 ± 1.6238 pg/mL; $P = .0623$) and 12 months (0.051 ± 0.7182 pg/mL; $P = .0625$). However, in the placebo arm, the values decreased compared with baseline at 12 months (−0.023 ± 6.3325 pg/mL; $P = .0156$). The mean difference between the 2 groups at 12-month follow-up was 0.28 pg/mL (95% CI, −1.48 to −2.03), which was not significant ($P = .4546$).

C-Terminal Cross-Linked Telopeptide of Type II Collagen. Analysis of the data was performed using the generalized estimating equation method, which showed that in the BMMSC arm, there was no significant change in values compared with the placebo arm, irrespective of time (7.79 ng/mmol; $P = .863$).

DISCUSSION

Despite the enormous burden of osteoarthritis, there are no disease-modifying drugs that have demonstrated consistent efficacy or have been approved for use worldwide. This may be because of the heterogeneity of the disease process, as it makes it difficult to target different pathways for a pharmacological intervention. Because of the inconsistency of palliative treatment, the role of MSC therapy

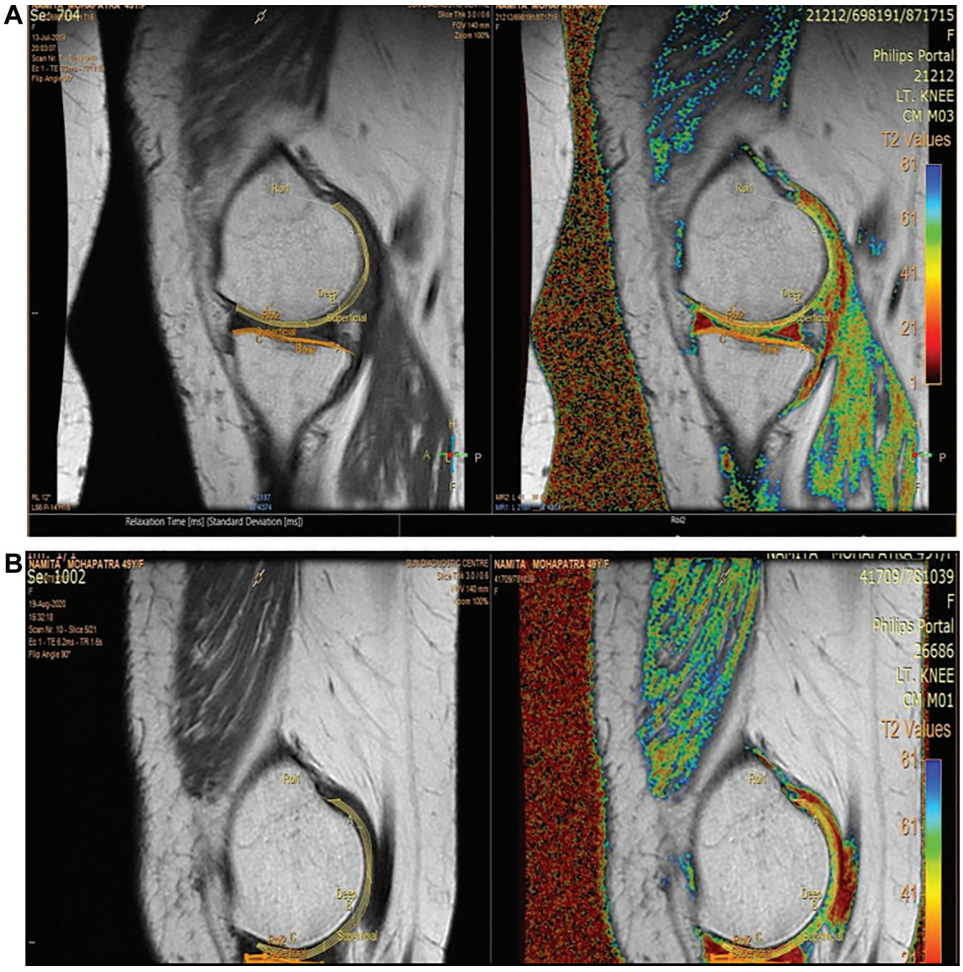

Figure 5. Representative images of T2 mapping at (A) baseline and (B) 12 months of bone marrow–derived mesenchymal stromal cell therapy. Decreasing T2 values correspond to an improvement in hydration and the collagen network of cartilage. There was a decrease in the relaxation time of deep cartilage from 56.27 ms at baseline to 44.53 ms at 12-month follow-up.

has come to the forefront to provide a potential disease-modifying approach for the regeneration of damaged articular cartilage in addition to symptomatic improvement. Studies have shown that an intra-articular injection of MSCs improved pain, stiffness, and function of the affected joint.[8,11,21] In this study, both the VAS and the WOMAC total scores improved by 41.58% and 46.60%, respectively, at 12-month follow-up compared with the placebo arm. Similarly, WOMAC subscores showed a statistically significant improvement at 12-month follow-up. The WOMAC and VAS are important PROMs, which are commonly used in clinical research for the evaluation of treatment effects.[35]

The interpretation of PROMs is challenging, as statistically significant differences may not always be clinically meaningful changes. Hence, the concept of the MCID has emerged for evaluating the changes in the PROM score from the patient perspective.[2] In previously published results, an MCID of $\geq$20% between the study groups is an indication of the effectiveness of the WOMAC and its subscores.[1,34] In our study, 89.2% of the patients injected with BMMSCs showed an improvement of $\geq$20% in the WOMAC total score at 12-month follow-up compared with 26.5% of patients in the placebo arm. Another published study[9] using stromal vascular fraction in knee osteoarthritis found an MCID of 33% in the WOMAC total score at 6-month follow-up. In a published review, Prodromos et al[32] examined the efficacy of autologous MSCs in osteoarthritis and showed that an MSC injection provided a consistent improvement compared with placebo.

In our study, the VAS score showed an improvement of $\geq$20% in 89.2% of patients in the BMMSC arm, which matched the value of improvement for the VAS (28.7 units) in a published review that used autologous MSCs.[32] This demonstrates the potential of BMMSCs to provide prolonged symptomatic relief for $\geq$1 year.

There is a debate regarding the use of autologous versus allogenic MSCs for efficacy in osteoarthritis. Autologous MSCs have the advantage of potentially minimizing the immune response and may have better efficacy. Further, the quantity of cells that can be obtained during a single harvest as well as the potential time for expansion should

be considered. However, disadvantages include the fact that as most of the patients are elderly, the differentiation and proliferation potential of the cells may be compromised. Certain metabolic conditions such as diabetes and obesity can predispose the differential potential of these cells toward the adipogenic lineage rather than chondrogenic differentiation.[7,26] Also, the proliferative capacity of the cells decreases with age.[4] Finally, a procedure of some type is required to harvest the donor cells, exposing patients to discomfort.

The use of allogenic cells offers certain advantages, as they can be used "off the shelf" because they can be banked, are available on demand, and can be transported with minimal delay. As these cells are extracted from healthy, young donors, the risk of a decreased regenerative potential is minimized. Further, these cells guarantee quality control and reduce the cost of cell therapies. However, there may be a risk of immunological incompatibility; because of low or modest levels of major histocompatibility complex class I molecules and the lack of expression of major histocompatibility complex class II and costimulatory molecules, this risk is minimized.[23] Recently, many published studies have used allogenic MSCs in osteoarthritis and reported no AEs that were related to the study drug, thus concluding that allogenic MSCs are safe and efficacious.[11,19,21,27,31] These findings are supported by the results of this study.

We analyzed the potency of BMMSCs by their ability to secrete prochondrogenic factors such as TSP-2. As osteoarthritis is a degenerative disease associated with the loss of cartilage, we believe that the presence of these molecules in the secretome of MSCs affect the clinical outcome. TSP-2 is an extracellular matrix protein and plays a major role in determining the chondrogenic differentiation potential of MSCs[16] and also stimulates the differentiation of endogenous chondroprogenitor cells.[17] In our analysis, we demonstrated that all the batches of BMMSCs secreted a steady level of TSP-2 at the 72-hour time point in culture.

There is an ongoing debate that repeated injections of MSCs may have better efficacy compared with a single injection. In a study using umbilical cord MSCs, the authors showed that at the end of 12-month follow-up, the repeated injection group (20 million cells every 6 months) experienced a significant improvement in the WOMAC total score, WOMAC pain subscore, and VAS score compared with the control group.[27] In another dose-finding study (10 million, 20 million, and 50 million of adipose tissue–derived MSCs), the patients in each dose group received 2 injections at 3 and 6 weeks after liposuction, and subsequently, a third injection was given after 48 weeks.[39] It was seen that after the first 2 injections, there was a substantial improvement in pain, function, and quality of life until the 12th week; thereafter, a decreasing trend was observed. Yet, after the third injection, the improvement rate increased further, thus highlighting a time- and dose-dependent effect.[39] In our study, a single intra-articular injection of pooled, cultured MSCs showed a prolonged improvement up to 1 year. This may be caused by the enhanced anti-inflammatory potential, better immunomodulatory properties, and higher secretion of chondrogenic factors in these pooled cells, which create an optimum environment for a controlled regenerative pathway in the affected joint. Pooling of cells from $\geq$2 donors potentially helps to compensate for the variability between the donors and balances the various properties of the different donor cell populations, thus increasing the efficacy of these pooled cells. The dose dependency and frequency of allogenic injections require further study.

T2 mapping of MRI, which uses the T2 relaxation time as a parameter, provides both quantitative and qualitative analyses to observe early changes within cartilage and assess damage and the regenerative response.[4,28] T2 relaxation time is a function of hydration, collagen content, and collagen fibril orientation in the extracellular matrix. Hence, a longer T2 relaxation time is seen in cartilage with osteoarthritis, whereas healing will decrease the relaxation time in affected areas.[6] In a published study, the use of allogenic BMMSCs (40 million cells) significantly improved cartilage quality as seen on T2 mapping, suggesting that MSCs aid in cartilage repair and regeneration[42] at 1-year follow-up. In another study by the same research group, using autologous, expanded BMMSCs with the same dose showed an improvement in cartilage quality using T2 relaxation time in 11 of 12 patients injected.[29] Yet, a study by Lee et al,[22] using adipose tissue–derived MSCs (100 million cells), showed no significant improvement in cartilage quality at 6-month follow-up in the cell group, whereas the defect in the control group increased. In our study, at 1-year follow-up, cartilage quality was maintained in the deep cartilage of the medial compartment (the most commonly affected in osteoarthritis), with a mean T2 relaxation time of $<$38 ms in the BMMSC arm, whereas in the placebo arm, it increased to 47 ms, indicating the continuous degeneration of cartilage. As Orozco et al[29] showed a consistent improvement in cartilage quality over a 2-year follow-up period, it is expected that an improvement in cartilage quality can be seen over a longer time period.[22] In this study, cartilage volume increased by 34.07 units, although not significant, over a period of 1 year, and this is confirmed by similar findings in other studies.[18,24,42] CTX-II, a by-product of type II collagen breakdown and a biomarker for cartilage degradation, has been widely studied and is elevated in urine in patients with osteoarthritis,[33] thus indicative of disease progression. This biomarker is of high interest, as it has been shown to have increased sensitivity and analyte stability. Interestingly, in this study, the CTX-II level had a nonsignificant decrease by 7.79 ng/mmol in the BMMSC arm over a period of 1 year, which may have a chondroprotective effect, as reported earlier.[3] IL-10, an anti-inflammatory cytokine, has a chondroprotective effect, and it is involved in the synthesis of type II collagen and aggrecan.[14] This study has shown that IL-10 levels had a significant increase at 1-month follow-up, but the increase at 3 and 12 months was not significant in the BMMSC arm.

Our findings of the safety of BMMSCs are consistent with what is reported in the literature. Wang et al[44] conducted a meta-analysis of the past 15 years regarding the safety of both autologous and allogenic MSCs in 3546

participants and concluded that MSC administration is safe in all populations; related AEs were transient fever, administration site reactions (injection site bleeding, injection site swelling, injection site pain, injection site itching, and injection site infection), constipation, fatigue, and sleeplessness. We have reported injection site joint swelling and injection site pain events related to the study drug, which subsided after a few days with analgesics. No systemic side effects or SAEs related to the study drug were observed, thus confirming the safety profile of BMMSCs.

There are some limitations of the study. First, cartilage changes were identified by T2 mapping on MRI; however, arthroscopic and histological evaluations would have been the gold standard. Yet, these would have presented logistical and cost limitations as well as consent issues because of their invasive nature. Second, the dosing schedule is yet to be determined, as we only included a single-dose group. However, we found that even a single dose of BMMSCs had long-term efficacy for $\geq$1 year. Third, allogenic MSCs have the risk of immunological incompatibility, which is minimized because of the inherent characteristics of these cells. Yet, to confirm the presence or absence of incompatibility measurements of a panel-reactive antibody is a good parameter—which, however, was not done in this study. Fourth, the biodistribution of the cells to assess the nature and extent of distribution, tissue engraftment, and differentiation of cells was not examined, with this being a blinded study and the difficulties of doing so in patients because of ethical considerations. Fifth, the study will continue for 2 years (1-year data presented here), and it will be important to obtain long-term follow-up data of these patients to determine durability and safety. Finally, for future studies, it may be interesting to observe the safety and efficacy of genetically modified MSCs overexpressing IL-10 and transforming growth factor–beta in osteoarthritis.

CONCLUSION

Allogenic, cultured, and pooled BMMSCs were safe and effective for the treatment of grade 2 and 3 osteoarthritis. The intervention provided sustained relief of pain and stiffness and improved physical function at 12-month follow-up.

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REFERENCES

1. Bellamy N, Bell MJ, Goldsmith CH, et al. Evaluation of WOMAC 20, 50, 70 response criteria in patients treated with Hylan G-F 20 for knee osteoarthritis. *Ann Rheum Dis*. 2005;64(6):881-885.
2. Çelik D, Çoban Ö, Kılıçoğlu Ö. Minimal clinically important difference of commonly used hip-, knee-, foot-, and ankle-specific questionnaires: a systematic review. *J Clin Epidemiol*. 2019;113:44-57.
3. Chahal J, Gómez-Aristizábal A, Shestopaloff K, et al. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. *Stem Cells Transl Med*. 2019;8(8):746-757.
4. Dexheimer V, Mueller S, Braatz F, Richter W. Reduced reactivation from dormancy but maintained lineage choice of human mesenchymal stem cells with donor age. *PLoS One*. 2011;6(8):e22980.
5. Diekman BO, Guilak F. Stem cell-based therapies for osteoarthritis: challenges and opportunities. *Curr Opin Rheumatol*. 2013;25(1):119-126.
6. Dunn TC, Lu Y, Jin H, Ries MD, Majumdar S. T2 relaxation time of cartilage at MR imaging: comparison with severity of knee osteoarthritis. *Radiology*. 2004;232(2):592-598.
7. Ferone A, Messina G. Sera of overweight patients alter adipogenesis and osteogenesis of bone marrow mesenchymal stromal cells, a phenomenon that also persists in weight loss individuals. *Stem Cell Res Ther*. 2016;6(7):347. doi:0.4172/2157-7633.1000347
8. Garay-Mendoza D, Villarreal-Martínez L, Garza-Bedolla A, et al. The effect of intra-articular injection of autologous bone marrow stem cells on pain and knee function in patients with osteoarthritis. *Int J Rheum Dis*. 2017;21(1):140-147.
9. Garza JR, Campbell RE, Tjoumakaris FP, et al. Clinical efficacy of intra-articular mesenchymal stromal cells for the treatment of knee osteoarthritis: a double-blinded prospective randomized controlled clinical trial. *Am J Sports Med*. 2020;48(3):588-598.
10. 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.
11. Gupta PK, Chullikana A, Rengasamy M, et al. Efficacy and safety of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells (Stempeucel®): preclinical and clinical trial in osteoarthritis of the knee joint. *Arthritis Res Ther*. 2016;18(1):301.
12. Gupta PK, Dutta S, Kala S, et al. Phase IV postmarketing surveillance study shows continued efficacy and safety of Stempeucel in patients with critical limb ischemia due to Buerger's disease. *Stem Cells Transl Med*. 2021;10(12):1602-1613.

13. Gupta PK, Krishna M, Chullikana A, Desai S, Murugesan R, Dutta S. 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. *J Vasc Surg*. 2017;66(4):1303.
14. Iannone F. Increased expression of nerve growth factor (NGF) and high affinity NGF receptor (p140 TrkA) in human osteoarthritic chondrocytes. *Rheumatology*. 2002;41(12):1413-1418.
15. Indian Council of Medical Research, Department of Biotechnology. National guidelines for stem cell research, https://dbtindia.gov.in/sites/default/files/National_Guidelines_StemCellResearch-2017.pdf
16. Jeong SY, Ha J, Lee M, et al. Autocrine action of thrombospondin-2 determines the chondrogenic differentiation potential and suppresses hypertrophic maturation of human umbilical cord blood-derived mesenchymal stem cells. *Stem Cells*. 2015;33(11):3291-3303.
17. Jeong SY, Kim DH, Ha J, et al. Thrombospondin-2 secreted by human umbilical cord blood-derived mesenchymal stem cells promotes chondrogenic differentiation. *Stem Cells*. 2013;31(10):2136-2148.
18. Jo CH, Chai JW, Jeong EC, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a 2-year follow-up study. *Am J Sports Med*. 2017;45(12):2774-2783.
19. Khalifeh Soltani S, Forogh B, Ahmadbeigi N, et al. Safety and efficacy of allogenic placental mesenchymal stem cells for treating knee osteoarthritis: a pilot study. *Cytotherapy*. 2019;21(1):54-63.
20. Kolasinski SL, Neogi T, Hochberg MC, et al. 2019 American College of Rheumatology/Arthritis Foundation guideline for the management of osteoarthritis of the hand, hip, and knee. *Arthritis Care Res*. 2020;72(2):149-162.
21. Kuah D, Sivell S, Longworth T, et al. Safety, tolerability and efficacy of intra-articular Progenza in knee osteoarthritis: a randomized double-blind placebo-controlled single ascending dose study. *J Transl Med*. 2018;16(1):49.
22. Lee W-S, Kim HJ, Kim K-I, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: a phase IIb, randomized, placebo-controlled clinical trial. *Stem Cells Transl Med*. 2019;8(6):504-511.
23. Lohan P, Treacy O, Griffin MD, Ritter T, Ryan AE. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells and their extracellular vesicles: are we still learning? *Front Immunol*. 2017;8:1626.
24. Lu L, Dai C, Zhang Z, et al. Treatment of knee osteoarthritis with intra-articular injection of autologous adipose-derived mesenchymal progenitor cells: a prospective, randomized, double-blind, active-controlled, phase IIb clinical trial. *Stem Cell Res Ther*. 2019;10(1):143.
25. Mamidi MK, Nathan KG, Singh G, et al. Comparative cellular and molecular analyses of pooled bone marrow multipotent mesenchymal stromal cells during continuous passaging and after successive cryopreservation. *J Cell Biochem*. 2012;113(10):3153-3164.
26. Mansilla E, Díaz Aquino V, Zambón D, et al. Could metabolic syndrome, lipodystrophy, and aging be mesenchymal stem cell exhaustion syndromes? *Stem Cells Int*. 2011;2011:943216.
27. Matas J, Orrego M, Amenabar D, et al. Umbilical cord-derived mesenchymal stromal cells (MSCs) for knee osteoarthritis: repeated MSC dosing is superior to a single MSC dose and to hyaluronic acid in a controlled randomized phase I/II trial. *Stem Cells Transl Med*. 2019;8(3):215-224.
28. Mosher TJ, Dardzinski BJ. Cartilage MRI T2 relaxation time mapping: overview and applications. *Semin Musculoskelet Radiol*. 2004;8(4):355-368.
29. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells. *Transplantation*. 2013;95(12):1535-1541.
30. Pal CP, Singh P, Chaturvedi S, Pruthi KK, Vij A. Epidemiology of knee osteoarthritis in India and related factors. *Indian J Orthop*. 2016;50(5):518-522.
31. Park Y-B, Ha C-W, Lee C-H, Yoon YC, Park Y-G. Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. *Stem Cells Transl Med*. 2017;6(2):613-621.
32. Prodromos C, Finkle S, Rumschlag T, Lotus J. Autologous mesenchymal stem cell treatment is consistently effective for the treatment of knee osteoarthritis: the results of a systematic review of treatment and comparison to a placebo group. *Medicines (Basel)*. 2020;7(8):42.
33. Qvist P, Bay-Jensen A-C, Christiansen C, Sondergaard BC, Karsdal MA. Molecular serum and urine marker repertoire supporting clinical research on joint diseases. *Best Pract Res Clin Rheumatol*. 2011;25(6):859-872.
34. Raynauld JP, Torrance GW, Band PA, et al. A prospective, randomized, pragmatic, health outcomes trial evaluating the incorporation of Hylan G-F 20 into the treatment paradigm for patients with knee osteoarthritis (part 1 of 2): clinical results. *Osteoarthritis Cartilage*. 2002;10(7):506-517.
35. Rolfson O, Bohm E, Franklin P, et al. Patient-reported outcome measures in arthroplasty registries report of the Patient-Reported Outcome Measures Working Group of the International Society of Arthroplasty Registries, part II: recommendations for selection, administration, and analysis. *Acta Orthop*. 2016;87(suppl 1):9-23.
36. Ronn K, Reischl N, Gautier E, Jacobi M. Current surgical treatment of knee osteoarthritis. *Arthritis*. 2011;2011:454873.
37. Shane Anderson A, Loeser RF. Why is osteoarthritis an age-related disease? *Best Pract Res Clin Rheumatol*. 2010;24(1):15-26.
38. Singh A, Antony B. Response to the letter to editor by Chen et al. Regarding: burden of osteoarthritis in India and its states, 1990-2019. Findings from the Global Burden of Disease Study 2019. *Osteoarthritis Cartilage*. 2022;30(10):1413-1414.
39. Song Y, Du H, Dai C, et al. Human adipose-derived mesenchymal stem cells for osteoarthritis: a pilot study with long-term follow-up and repeated injections. *Regen Med*. 2018;13(3):295-307.
40. Thambiah MD, Tan MKL, Hui JHP. Role of high tibial osteotomy in cartilage regeneration: is correction of malalignment mandatory for success? *Indian J Orthop*. 2017;51(5):588-599.
41. van Buul GM, Villafuertes E, Bos PK, et al. Mesenchymal stem cells secrete factors that inhibit inflammatory processes in short-term osteoarthritic synovium and cartilage explant culture. *Osteoarthritis Cartilage*. 2012;20(10):1186-1196.
42. Vega A, Martín-Ferrero MA, Del Canto F, et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells. *Transplantation*. 2015;99(8):1681-1690.
43. Wang M. Generalized estimating equations in longitudinal data analysis: a review and recent developments. *Adv Stat*. 2014;2014:1-11.
44. Wang Y, Yi H, Song Y. The safety of MSC therapy over the past 15 years: a meta-analysis. *Stem Cell Res Ther*. 2021;12(1):545.
45. Xie F, Thumboo J, Fong KY, et al. A study on indirect and intangible costs for patients with knee osteoarthritis in Singapore. *Value Health*. 2008;11(suppl 1):S84-S90.