Injectable Cultured Bone Marrow—Derived Mesenchymal Stem Cells in Varus Knees With Cartilage Defects Undergoing High Tibial Osteotomy: A Prospective, Randomized Controlled Clinical Trial With 2 Years' Follow-up

Keng Lin Wong, M.R.C.S.(Edin), Kevin Boon Leng Lee, F.R.C.S.(Edin, Orth), Bee Choo Tai, Ph.D.(Stats), Ping Law, Ph.D., Eng Hin Lee, M.D., and James H. P. Hui, F.R.C.S.(Edin), F.A.M.S.(Sing), M.D.

Purpose: To analyze the results of the use of intra-articular cultured autologous bone marrow—derived mesenchymal stem cell (MSC) injections in conjunction with microfracture and medial opening-wedge high tibial osteotomy (HTO). **Methods:** Fifty-six knees in 56 patients with unicompartmental osteoarthritic knees and genu varum were randomly allocated to the cell-recipient group (n = 28) or control group (n = 28). Patients who had a joint line congruity angle of more than 2°, malalignment of the knee from femoral causes, a fixed flexion deformity, or age older than 55 years were excluded. All patients underwent HTO and microfracture. The cell-recipient group received intra-articular injection of cultured MSCs with hyaluronic acid 3 weeks after surgery, whereas the control group only received hyaluronic acid. The primary outcome measure was the International Knee Documentation Committee (IKDC) score at intervals of 6 months, 1 year, and 2 years postoperatively. Secondary outcome measures were Tegner and Lysholm clinical scores and 1-year postoperative Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores. **Results:** The median age of the patients was 51 years, with a mean body mass index of 23.85. Both treatment arms achieved improvements in Tegner, Lysholm, and IKDC scores. After adjustment for age, baseline scores, and time of evaluation, the cell-recipient group showed significantly better scores. The effect of treatment showed an added improvement of 7.65 (95% confidence interval [CI], 3.04 to 12.26; $P = .001$) for IKDC scores, 7.61 (95% CI, 1.44 to 13.79; $P = .016$) for Lysholm scores, and 0.64 (95% CI, 0.10 to 1.19; $P = .021$) for Tegner scores. Magnetic resonance imaging scans performed 1 year after surgical intervention showed significantly better MOCART scores for the cell-recipient group. The age-adjusted mean difference in MOCART score was 19.6 (95% CI, 10.5 to 28.6; $P < .001$). **Conclusions:** Intra-articular injection of cultured MSCs is effective in improving both short-term clinical and MOCART outcomes in patients undergoing HTO and microfracture for varus knees with cartilage defects. **Level of Evidence:** Level II, randomized controlled trial.

Cartilage erosion is a major feature in osteoarthritis (OA). For large articular defects in patients with OA, repair methods are limited, and many elderly patients undergo a total knee replacement for severely damaged joints. In younger patients with less damaged joints, such as joints with medial unicompartmental OA, many attempts have been made to promote cartilage regeneration in the hope of delaying the need for a total knee replacement.

For medial unicompartmental OA with varus deformity, medial opening high tibial osteotomy (HTO) has been shown to be an effective treatment option with good clinical results.[1,2] It effectively unloads the medial compartment and allows for healing of the articular cartilage. Akizuki et al.[3] have shown that marrow stimulation procedures performed in conjunction with

From the Department of Orthopaedic Surgery, University Orthopaedic, Hand and Reconstructive Microsurgery Cluster (K.L.W., K.B.L.L., E.H.L., J.H.P.H.), and Tissue Engineering and Cell Therapy (GMP) Laboratory (P.L.), National University Health System, and Saw Swee Hock School of Public Health, National University of Singapore (B.C.T.), Singapore.

The authors report that they have no conflicts of interest in the authorship and publication of this article.

Received May 8, 2013; accepted September 18, 2013.

Address correspondence to James H. P. Hui, F.R.C.S.(Edin), F.A.M.S.(Sing), M.D., Division of Paediatric Orthopaedic Surgery, Department of Orthopaedic Surgery, University Orthopaedic, Hand and Reconstructive Microsurgery Cluster, National University Hospital, 1E Kent Ridge Road, NUHS Tower Block, Level 11, 119228, Singapore. E-mail: james_hui@nuhs.edu.sg

0749-8063/13317/$36.00

http://dx.doi.org/10.1016/j.arthro.2013.09.074

HTO can promote chondral repair. However, marrow stimulation alone has been shown to stimulate growth of fibrocartilage and yield decreases in clinical scores after 24 months in some studies.[4,5] In a trial conducted in 2002, Wakitani et al.[6] showed that 24 patients who underwent dome HTO and were also treated with cultured autologous bone marrow–derived mesenchymal stem cell (MSC) transplantation with a gel-cell composite had better chondral healing than patients who underwent HTO alone. In an equine model, McIlwraith and colleagues[7] were able to show significant arthroscopic and immunohistologic improvements when MSCs were administered by intra-articular injections to augment healing with microfracture as compared with microfracture alone.

Thus a prospective, randomized clinical trial was designed to analyze the results of the use of intra-articular cultured autologous bone marrow–derived MSC injections in conjunction with microfracture and a medial opening-wedge HTO. We hypothesized that the use of cultured injectable MSCs with microfracture and HTO would result in better cartilage healing and improved clinical outcomes.

Methods

Patient Selection

This prospective, randomized clinical trial was approved by the local ethics committee. The trial is ongoing and still recruiting patients. Informed consent was obtained from all the patients. Inclusion criteria were medial-compartment OA with genu varum in patients aged younger than 55 years diagnosed either arthroscopically and/or radiologically, normal lateral joint space, no fixed flexion deformity of the knee, and no collateral ligament instability. Exclusion criteria were a joint line congruity angle more than 2°, malalignment of the knee from femoral causes, and bicompartmental and tri-compartmental osteoarthritis. Patients who were unable to tolerate magnetic resonance imaging (MRI) scans, unable to answer subjective questionnaires, or not mentally fit to provide informed consent were also excluded.

Patients were analyzed preoperatively based on weight-bearing anteroposterior and non–weight-bearing lateral radiographs of the involved knee, along with standing long leg films, to confirm medial OA and to exclude any significant patellofemoral and lateral-compartment OA. The degree of correction to be achieved was calculated, aiming to bring the mechanical axis to the Fujisawa point postoperatively. Patients were enrolled in the study by the principal investigator of the trial. Patients were randomized into 2 groups: the cell-recipient group or the control group. Simple randomization methods were used, in which each patient, when enrolled in the trial, was asked to choose either of 2 identical envelopes with either the control or treatment group indicated inside. The randomization process was conducted by a hospital staff member blinded to the patients' data. Patients, however, were not blinded in terms of intervention method. A total of 56 patients were enrolled, with 28 knees comprising the cell-recipient group and 28 knees making up the control group.

Surgical Procedure

All patients underwent arthroscopy and microfracture followed by medial opening HTO fixed with a long fixed-angle locking plate. Surgery was performed with patients under general anesthesia and with tourniquet control. All patients underwent arthroscopy to grade the cartilage status based on the International Cartilage Repair Society (ICRS) classification.[8] The size, number, and stage of chondral ulcers were documented. The size of the lesion was determined by direct measurement of the lesion arthroscopically with a standardized tool (right-angle probe with a length of 5 mm). Marrow stimulation was achieved by performing microfracture as described by Steadman et al.[9] After wound closure, while patient was under anesthesia, bone marrow was harvested from the iliac crest of the contralateral hip.

Bone Marrow–Derived MSC Preparation

Autologous MSCs were cultured from bone marrow in a class B clean room environment. The culture step is common practice to generate MSCs because the marrow aspirate contains a mixture of different red blood cells, platelets, and leukocytes (a majority consists mostly of neutrophils, lymphocytes, and monocytes).[10] MSC and MSC precursors are extremely rare under normal conditions in human marrow (<0.05% of low-density mononuclear cells) before culture.[11] In brief, between 35 and 74 mL of marrow (median, 49 mL) was collected from the hip of the patients under general anesthesia during microfracture surgery in the operating theater. Uncoagulated whole blood was also collected (from which autologous serum was prepared and stored at −20°C). Most of the red blood cells were removed from marrow by dextran sedimentation, and the leukocyte-rich fraction was washed and cultured for MSCs in Dulbecco's Modified Eagle Medium plus 10% fetal bovine serum and ascorbic acid (50 μg/mL) (all from Invitrogen, Paisley, Scotland) in 5 to 6 T-75 flasks. Culture medium was completely replaced every 2 to 3 days until harvest. MSCs underwent passaging (p0 to p1) (into the same number of flasks) when they reached greater than 30% confluency (usually after 10 to 13 days; median, 12 days) by treating with trypsin (Invitrogen) and washes. Between 9 and 11 days, culture supernatant was sent for assays for mycoplasma and microbial contamination (aerobic and anaerobic bacteria, as well as fungus). MSC release criteria (for administration) include the following: no growth on

mycoplasma and microbial culture, confluency greater than 70%, normal MSC morphology, and greater than 75% viable (by fluorescent acridine orange and propidium iodide staining). Between 19 and 23 days (median, 22 days), MSCs were trypsinized, washed, and resuspended in 0.5 to 1 mL of autologous serum and delivered to the clinic for administration directly into the knee within 4 hours. All patients received the same volume regardless of the total number of MSCs. A small fraction from each sample was removed before resuspension in serum to assess the cell count, viability, and flow cytometry (MSC phenotyping kit; Miltenyi Biotec, Bergisch Gladbach, Germany) using FACS Canto II with FACSDiva software (BD, Franklin Lakes, NJ). All patients were administered p1 MSCs except one case in which the patient could not attend the appointment and hence p2 MSCs were used. The characteristics of the MSCs were as follows (mean ± standard deviation): total cells, $1.46 \pm 0.29 \times 10^7$; viability, 87.1% ± 6.4%; lineage negative (CD14, CD20, CD34, and CD45), 93.0% ± 5.5%; $CD73^+$, 95.2% ± 4.2%; $CD90^+$, 88.9% ± 6.7%; and $CD105^+$, 89.7% ± 6.3%. The panel of cell surface markers was chosen in accordance with the recommendations of the International Society for Cell Therapy as part of the MSC release criteria.[12]

Postoperative Rehabilitation Program and Follow-Up

Patients were mobilized non−weight bearing from the second postoperative day and were kept non−weight bearing for 6 weeks, after which they were allowed to bear weight as comfortable with a gradual return to full weight-bearing status. All patients were advised to perform regular continuous passive mobilization (CPM) for 8 hours per day, from the second postoperative day until 4 weeks postoperatively. The rate of CPM was balanced in both arms of the study group. This was made available during the inpatient stay and for rental once the patient was discharged. Range of motion started from 30° to 70° with gradual increments. Patients who were unable to be reimbursed for CPM would undergo physiotherapy training and be guided to complete 500 flexion and extension exercises per day. Thereafter patients underwent rehabilitation such as spinning on a stationary bicycle with no resistance to improve range of motion. Patients were reviewed on the 10th postoperative day for wound inspection, followed by another review at 3 weeks. During the second review, MSCs with 2 mL of hyaluronic acid (HA) were injected into the intra-articular space with a 19-gauge needle (under local anesthesia) for patients in the cell-recipient group, whereas the control group received HA alone under local anesthesia. Two more doses of 2 mL of HA injection were repeated at weekly intervals for all patients, per the manufacturer's instructions for dosage and administration. Once full weight bearing and good range of motion were achieved for the knee, the patient underwent a comprehensive rehabilitation program with the aim of restoring muscular function. This included the use of stationary bicycles, elliptical trainers, and treadmills, along with closed-chain exercises.

Patients were followed up at 6 weekly intervals thereafter for the first 6 months and then at 1-year and 2-year intervals. At the 6-month, 1-year, and 2-year reviews, Tegner and Lysholm knee scores and International Knee Documentation Committee (IKDC) scores were documented for all patients. MRI was performed after 1 year to look for cartilage regeneration based on the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scoring system.[13] The scoring instruments were chosen to evaluate both clinical and radiologic improvements. MRI was performed on a 1.5-T Signa HDxt MRI scanner (GE Medical Systems, Milwaukee, WI) with a dedicated extremity surface coil (HD TR knee PA). The normal knee sequences used were axial T2, coronal proton density fat saturated, coronal T1, sagittal proton density, and sagittal T2 fat saturated. The cartilage sequences used were axial SPGR (spoiled gradient recalled), sagittal SPGR, axial FIESTA (fast imaging employing steady-state acquisition), and sagittal FIESTA. Each MRI scan consists of 19 slices for the normal knee protocol and 32 slices for the cartilage sequences. All patients were positioned consistently with the joint space in the middle of the coil and the knee extended in the coil. MRI findings were evaluated by one of the authors blinded to the patients' data.

The primary outcome measure was clinical outcome with the IKDC scoring system. The secondary outcome measures were Tegner and Lysholm clinical scores and the MOCART scoring system.

Power Calculation and Statistical Analysis

We postulated a difference in mean IKDC score of 1.27 between treatments with an SD of 1.5. Then, with the assumption of a correlation between scores of 0.4 at the different time points (one before and 3 after randomization), a power of 95%, and a 2-sided test size of 5%, a minimum sample size of 20 per group, or 40 in total, would be required for this study.

The mixed-effects model (with random intercept) was used to evaluate the effect of treatment on the IKDC, Lysholm, and Tegner scores, adjusted for baseline score, age, and time of evaluation. This method of analysis appropriately accounts for the possible correlation between repeated measurements of an individual. Age-adjusted mean differences in total MOCART score at 1 year between treatment arms were evaluated by analysis of covariance. Comparisons of individual

Table 1. Patient Characteristics by Treatment

	HTO + MSCs (n = 28)	HTO (n = 28)	All Patients (N = 56)
Age (yr)			
Median	53	49	51
Range	36-54	24-54	24-54
Sex (%)			
Male	15 (46)	14 (50)	27 (48)
Female	13 (54)	14 (50)	29 (52)
Body mass index			
Mean	23.81	23.89	23.85
SD	2.17	3.20	2.30
Tegner before injury (%)			
0-2	0 (0)	0 (0)	0 (0)
3-5	19 (68)	18 (64)	37 (66)
≥6	9 (32)	10 (36)	19 (34)
Tegner score at baseline (%)			
0-2	15 (54)	16 (57)	31 (55)
3-5	13 (46)	11 (39)	24 (43)
≥6	0 (0)	1 (4)	1 (2)
Lysholm score at baseline			
Mean	41.9	50.4	46.2
SD	19.2	23.0	21.4
IKDC score at baseline			
Mean	33.9	36.0	34.9
SD	11.4	13.7	12.5
Intraoperative ICRS grade (%)			
2	5 (18)	3 (11)	8 (14)
3	8 (29)	8 (29)	16 (29)
4	15 (54)	17 (61)	32 (57)
Primary lesion size (cm^2)			
Median	6.0	3.5	5.0
SD	2.8-12.0	1.5-6.8	1.5-9.3
Preoperative varus angulation (°)			
Mean	9.0	8.6	8.8
SD	2.3	1.6	2.0

MOCART scores between treatment groups were made by use of the Fisher test. All statistical assessments were made assuming a 2-sided test at the conventional 5% level of significance with Stata software, version 12 (StataCorp, College Station, TX).

Results

Patient Characteristics

The patient demographic data and characteristics are shown in Table 1. Figure 1 reports the trial profile of this study. There were 56 patients recruited in the study, with 28 eligible patients in each arm. There was no refusal of participation or consent. There was no dropout during the study. All patients completed 2 years of follow-up and were compliant with the treatment options prescribed. The MSC group had a mean of 24.8 months (range, 24 to 36 months) of follow-up; the control group had a mean of 24.5 months (range, 24 to 35 months) of follow-up (Table 1). The median age of the MSC group was older, at 53 years, as compared with the control group, whose median age was 49 years. Most patients had severe chondral defects, with 57% of patients having ICRS stage 4 chondral damage (Table 1). All primary lesions were located at the medial femoral condyle, and the median lesion size was 5.0 cm^2. The preinjury Tegner score was between 3 and 5 in 66% of the patients, with the remaining 34% having scores of 6 or greater (Table 1). The MSC group had marginally lower baseline IKDC and Lysholm scores than the control group. The median lesion size was larger, at 6.0 cm^2, in the cell-recipient group, as compared with the control group, at 3.5 cm^2 (Table 1). Other variables such as intraoperative ICRS grading and preoperative varus angulation were comparable between the 2 groups (Table 1).

Clinical Outcomes

The clinical outcomes of the patients are shown in Figs 2, 3, and 4, and the adjusted effect of treatment on the clinical scores is shown in Table 2. Both treatment arms achieved improvements in Tegner, Lysholm, and IKDC scores (Figs 2-4). After adjustment for age, baseline scores, and time of evaluation, the MSC group showed significantly better scores than the control group (Figs 2-4). The effect of treatment showed an added improvement of 7.65 (95% confidence interval [CI], 3.04 to 12.26; $P = .001$) for IKDC scores, 7.61 (95% CI, 1.44 to 13.79; $P = .016$) for Lysholm scores, and 0.64 (95% CI, 0.10 to 1.19; $P = .021$) for Tegner scores (Table 2).

We noted differences with respect to age, with the MSC group being notably older. The difference in baseline Lysholm score seemed appreciable. Thus we have adjusted for potential baseline differences in age and scores for all outcomes. The time of evaluation refers to the time effect. From the graphs (Figs 2-4), it is evident that the scores improved over time and hence we have accounted for this in the mixed model, by taking into account the increasing time trend. For the IKDC score, individual effects of age and baseline after adjustments were as follows: baseline, 0.55 (95% CI, 0.38 to 0.72; $P < .001$); age, −0.31 (95% CI, −0.55 to −0.06; $P = .013$). For the Lysholm score, individual effects of age and baseline after adjustments were as follows: baseline, 0.46 (95% CI, 0.33 to 0.59; $P < .001$); age, −0.20 (95% CI, −0.55 to 0.14; $P = .252$). For the Tegner score, individual effects of age and baseline after adjustments were as follows: baseline, 0.36 (95% CI, 0.18 to 0.54; $P < .001$); age, −0.03 (95% CI, −0.06 to −0.005; $P = .023$). In summary, one unit increase in baseline score is associated with an increase in the IKDC score of 0.55, Lysholm score of 0.46, and Tegner score of 0.36. There is an inverse association between scores and age.

There were no deep infections of implants, periprosthetic fractures, or any other serious adverse events reported during the duration of the study.

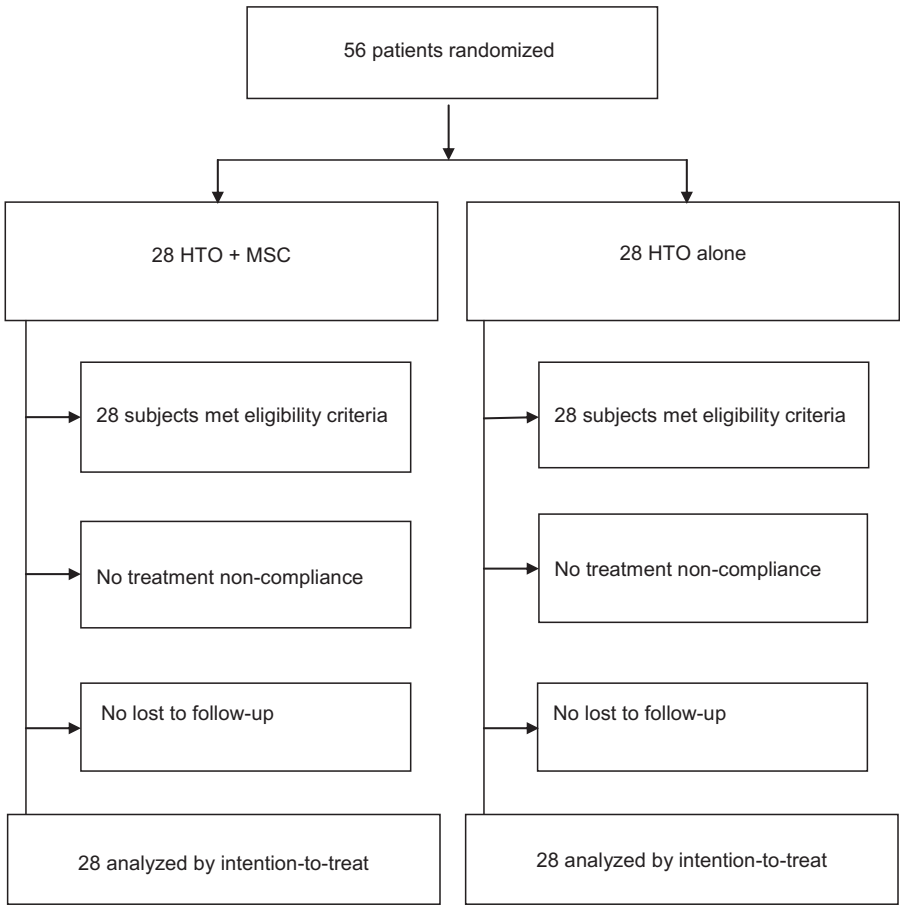

Fig 1. Trial profile of all patients randomized in study. The patients were equally randomized into 2 groups of 28 subjects each. No patients were lost to follow-up during the 2-year period, and all were compliant with the allocated mode of treatment. (HTO, control group [high tibial osteotomy and HA]; MSC + HTO, mesenchymal stem cell group [high tibial osteotomy, MSCs, and HA]).

Imaging Evaluation of Cartilage Repair

MRI scans carried out 1 year after surgical intervention showed significantly better MOCART scores for the MSC group when compared with the control group. The age-adjusted mean difference in total MOCART score was 19.6 (95% CI, 10.5 to 28.6; $P < .001$) (Table 3).

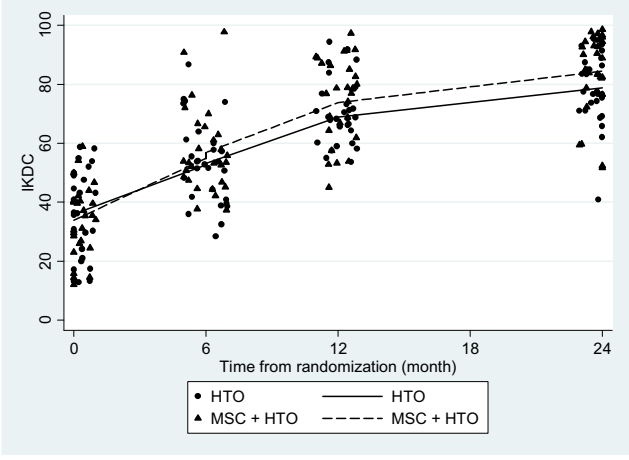

Fig 2. IKDC scores of both groups after 24 months. After adjustment for age, baseline scores, and time of evaluation, the effect of treatment showed an added improvement of 7.65 (95% CI, 3.04 to 12.26; $P = .001$) for the MSC group. (HTO, control group [high tibial osteotomy and HA]; MSC + HTO, mesenchymal stem cell group [high tibial osteotomy, MSCs, and HA]).

The MOCART scores were significantly better in the MSC group, with 9 patients (32%) who had complete cartilage coverage of their lesions versus none in the control group (Table 3). In the MSC group, 10 patients (36%) had greater than 50% cartilage coverage, which was significantly higher than in the control group, in which only 4 patients (14%) had similar results (Table 3). Significantly better integration of the regenerated cartilage was found in 61% of patients in the MSC group, with complete integration of the regenerated cartilage to the border zone, whereas most of the control group (86%) showed incomplete integration with visible defects (Table 3). Figure 5 shows examples of the MRI scans used in the evaluation of our patients.

Discussion

The adult stem cell fraction is present in nucleated cells of the marrow; however, very few are actually MSCs capable of differentiating into bone, cartilage, or muscle.[14] The use of stem cells with their chondrogenic potential has been well documented in both in vitro and in vivo studies.[15,16] This study provides a novel method of cartilage repair for genu varum knees, which

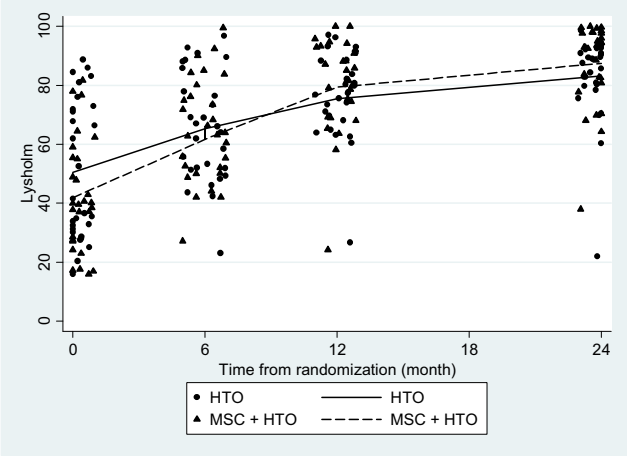

Fig 3. Lysholm scores of both groups after 24 months. The difference in baseline Lysholm score is evident, with a lower Lysholm score reported in the MSC group. After adjustment for age, baseline scores, and time of evaluation, the effect of treatment showed an added improvement of 7.61 (95% CI, 1.44 to 13.79; $P = .016$) for the MSC group. (HTO, control group [high tibial osteotomy and HA]; MSC, mesenchymal stem cell group [high tibial osteotomy, MSCs, and HA]).

uses intra-articular high-dose MSCs in HA medium combined with a medial HTO and microfracture. This was performed by a protocol that minimizes pain and anesthetic exposure. To date, we have not found any studies similar to this current method. In addition, this study used a prospective randomized study design with an equal number of patients in both the treatment and control arms. In this study we have proven that injectable cultured intra-articular MSCs are effective at improving both short-term clinical and MOCART

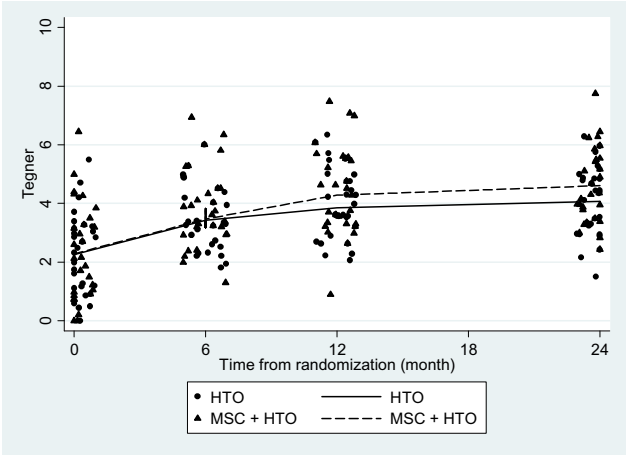

Fig 4. Tegner scores of both groups after 24 months. After adjustment for age, baseline scores, and time of evaluation, the effect of treatment showed an added improvement of 0.64 (95% CI, 0.10 to 1.19; $P = .021$) for the MSC group. (HTO, control group [high tibial osteotomy and HA]; MSC, mesenchymal stem cell group [high tibial osteotomy, MSCs, and HA]).

outcomes in patients with cartilage defects who undergo knee alignment surgery and microfracture.

The delivery of MSC injections suspended in HA has been proven to be viable in treating large cartilage defects in various animal models.[15,17] In an equine model, McIlwraith et al.[7] compared microfracture versus microfracture combined with MSCs and HA (20×10^6 cells) in the healing of 1-cm^2 defects on the medial femoral condyle. The study reported a significant increase in repair tissue firmness and a trend for better overall repair tissue quality by both arthroscopic and gross evaluation of knee cartilage. In the clinical setting, articular cartilage regeneration with injectable cultured autologous peripheral blood progenitor cells (PBPCs) and HA has shown good promise, with a recent clinical case series by Saw et al.[18] showing histologic evidence of hyaline cartilage regeneration after microfracture and PBPC injection. This study also provided patients with relatively pure MSCs (>85% by flow cytometry assessment of CD73, CD90, and CD105) at a dose of 10^7 cells, in contrast to the use of bone marrow or PBPC without culture, in which the MSC content was very low or undetermined.[11] This has been postulated to increase the likelihood of MSCs finding the site of cartilage injury themselves and attaching themselves at the site in need of repair.[19,20]

Wakitani et al.[6] reported improved chondral healing with MSC transplantation through a gel-cell composite, for which the patient has to undergo partial anesthesia for MSC harvesting by iliac crest methods. Thereafter the surgeon will have to access the knee through a medial parapatellar approach and suture the MSC composite to the remaining cartilage along with the main osteotomy, for which the patient undergoes a second session of anesthesia. We propose a less invasive and more convenient method, using arthroscopic microfracture and intra-articular injection of MSCs instead. Our protocol also allows the patient to have MSC harvesting under a single general anesthesia session along with the main surgery, thus significantly reducing the pain and anesthetic exposure in our patients.

Patients undergo HTO and microfracture to keep their activity levels satisfactory and prevent or delay the need for knee arthroplasty. A current literature review has reported improved survivorship when HTO is combined with microfracture, with statistics showing 91% survivorship at 7 years after the operation.[21] This combination also leads to a mean delay of 81.3 months before proceeding to knee arthroplasty.[21] Patient satisfaction rates after HTO and microfracture have been reported to be satisfactory up to 9 years.[21,22] However, microfracture may lead to regeneration of fibrous cartilage and may not be as robust as native articular hyaline cartilage, leading to poorer outcomes in terms of physical activity levels when compared with osteochondral autograft transfer.[6,23,24] Injection

Table 2. Effect of Treatment on IKDC, Lysholm, and Tegner Scores

	Unadjusted			Adjusted*		
Outcome	Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value
IKDC score	4.73	−1.31 to 10.77	.124	7.65	3.04 to 12.26	.001
Lysholm score	2.55	−4.93 to 10.02	.504	7.61	1.44 to 13.79	.016
Tegner score	0.45	−0.18 to 1.08	.158	0.64	0.10 to 1.19	.021

*Model adjusted for age, baseline score, and time of evaluation.

of MSCs into these knees further improves the survivability.

Limitations

The limitations of this study include short-term follow-up and the inability to blind the patients to their group assignment, which may lead to patient bias. The imbalance between the treatment arms with respect to age and baseline score despite a randomized study design could be accounted for by the simple randomization methods used in our study. A block randomization would ensure balance between the treatment arms. The patients in the current trial are continuing to be followed up at 5 years and 10 years after intervention, with further MRI and clinical scoring questionnaires prepared for patients to evaluate the midterm to long-term results. We were unable to blind the patients in this study because the harvesting of bone marrow from the iliac crest would have been evident to the patient after the surgery because of the presence of a wound and pain from the harvesting site.

In this study we were unable to standardize the size of the chondral lesions preoperatively; hence both groups were not homogeneous in terms of severity of cartilage

Table 3. Effect of Treatment on 1-Year MOCART Score

MOCART Score	HTO + MSCs	HTO	*P* Value
Total score			<.001
Mean*	62.32	43.21	
SD	17.56	13.55	
Degree of defect repair and filling of defect (%)			<.001
Complete (20)	9 (32)	0 (0)	
Hypertrophy (20)	0 (0)	0 (0)	
Incomplete, >50% of adjacent cartilage (10)	10 (36)	4 (14)	
Incomplete, <50% of adjacent cartilage (5)	6 (21)	21 (75)	
Subchondral bone exposed (0)	3 (11)	3 (11)	
Integration of border zone (%)			<.001
Complete (15)	17 (61)	4 (14)	
Incomplete, demarcating border visible (slit-like) (10)	3 (11)	0 (0)	
Incomplete, defect visible <50% of length (5)	4 (14)	15 (54)	
Incomplete, defect visible >50% of length (0)	4 (14)	9 (32)	
Surface of repair tissue (%)			.122
Surface intact (10)	8 (29)	2 (7)	
Surface damaged <50% of depth (5)	18 (64)	24 (86)	
Surface damaged >50% of depth (0)	2 (7)	2 (7)	
Structure of repair tissue (%)			.418
Homogeneous (5)	14 (50)	10 (36)	
Inhomogeneous (0)	14 (50)	18 (64)	
Signal intensity of repair tissue (%)			.111
Normal (identical to adjacent cartilage) (30)	1 (4)	0 (0)	
Nearly normal (slight areas of signal alteration) (15)	27 (96)	24 (86)	
Abnormal (large areas of signal alteration) (0)	0 (0)	4 (14)	
Subchondral lamina (%)			.014
Intact (5)	25 (89)	16 (57)	
Not intact (0)	3 (11)	12 (43)	
Subchondral bone (%)			>.999
Intact (5)	13 (46)	12 (43)	
Not intact (0)	15 (54)	16 (57)	
Adhesions (%)			.352
No (5)	27 (96)	24 (86)	
Yes (0)	1 (4)	4 (14)	
Effusion (%)			.669
No (5)	26 (93)	24 (86)	
Yes (0)	2 (7)	4 (14)	

*The age-adjusted mean difference in total MOCART score was 19.6 (95% CI, 10.5 to 28.6).

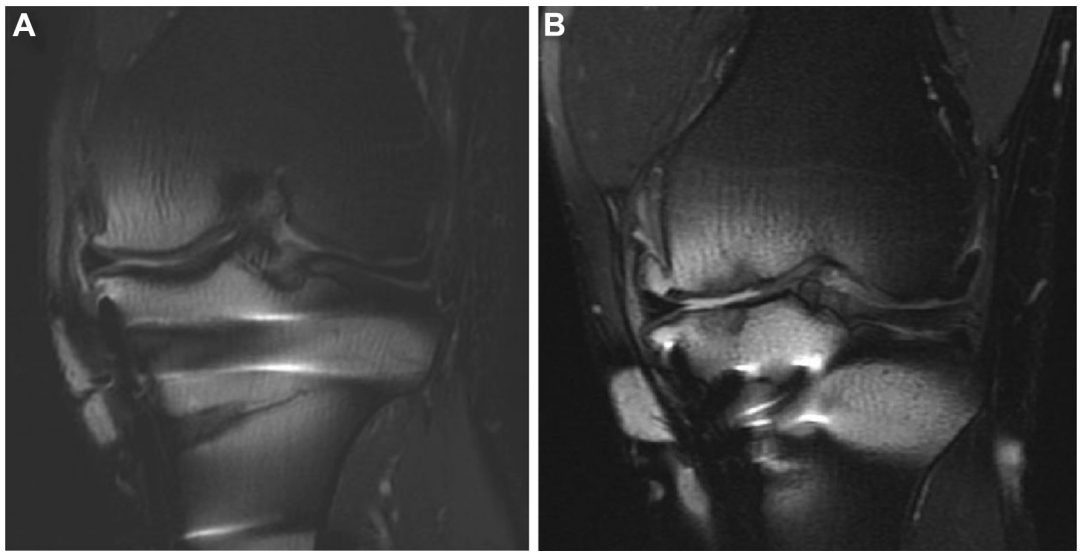

Fig 5. Magnetic resonance images of patients postoperatively. All patients were positioned consistently with the joint space in the middle of the coil and the knee extended in the coil in the supine position. The artifacts seen are caused by the proximal tibia locking plate used for the opening-wedge osteotomy. (A) MRI scan used for the MOCART score of a patient in the cell-recipient group. A coronal fast spin echo proton density fat-saturated image of the left knee is shown. MOCART scoring showed 85 points with complete repair filling and intact integration with an intact surface of cartilage. The subchondral lamina and bone at the medial tibia plateau show an altered structure. The artifacts seen are caused by the proximal tibia locking plate used for the opening-wedge osteotomy. (B) MRI scan used for the MOCART score of a patient in the control group. A coronal fast spin echo proton density fat-saturated image of the left knee is shown. MOCART scoring of the patient in the control group showed 15 points with minimal repair filling with subchondral bone exposed, poor integration of cartilage repair and subchondral lamina, and bone damage.

damage. This study reported a larger mean lesion size in the cell-recipient group of 6.0 cm^2 as compared with that in our control group, which measured 3.5 cm^2. The intraoperative ICRS classification between the 2 treatment arms, however, was comparable in severity. Despite a larger lesion size, better results were able to be produced by the cell-recipient group as compared with the control group, which further justifies the effectiveness of MSCs in this study.

The degree and magnitude of cartilage repair were not further evaluated by arthroscopic and histologic means in this study. Our patients did not undergo a routine preoperative MRI scan because we believe that cartilage imaging is best carried out under direct vision through arthroscopy before microfracture or MSC intervention. The study design did not include regular-interval surveillance arthroscopies and biopsies because we believed that this would affect patient safety with unnecessary anesthetic exposure and surgical risks.

In this study we were unable to further prove the exact mechanism of localization and homing of injectable MSCs to the desired regeneration site. We acknowledge the possibility that the MSCs may not have attached to the desired site in all cases. More clinical trials will be necessary to study the complex signaling mechanisms of MSCs when they are injected into the knee cartilage primed by microfracture. Kobayashi and colleagues[25-27] have demonstrated a novel cell delivery system using magnetically labeled MSCs and an external magnetic device for cartilage repairs. In vitro studies carried out by Kobayashi et al.[26] and animal studies carried out by Hori et al.[27] have shown great promise in using an external magnetic device to guide the MSCs to the area of cartilage defect, reporting histologic evidence of regeneration of native cartilage in areas of defects. Further clinical trials will be greatly beneficial in providing more evidence in patients regarding this method of localization and homing.

Conclusions

Intra-articular injection of cultured MSCs is effective in improving both short-term clinical and MOCART outcomes in patients undergoing HTO and microfracture for varus knees with cartilage defects.

References

1. Coventry MB, Ilstrup DM, Wallrichs SL. Proximal tibial osteotomy: A critical long-term study of eighty-seven cases. *J Bone Joint Surg Am* 1993;75:196-201.

2. Luites JW, Brinkman JM, Wymenga AB, van Heerwaarden RJ. Fixation stability of opening- versus closing-wedge high tibial osteotomy: A randomised clinical trial using radiostereometry. *J Bone Joint Surg Br* 2009;91:1459-1465.
3. Akizuki S, Yasukawa Y, Takizawa T. Does arthroscopic abrasion arthroplasty promote cartilage regeneration in osteoarthritic knees with eburnation? A prospective study of high tibial osteotomy with abrasion arthroplasty versus high tibial osteotomy alone. *Arthroscopy* 1997;13:9-17.
4. LaPrade RF, Bursch LS, Olson EJ, Havlas V, Carlson CS. Histologic and immunohistochemical characteristics of failed articular cartilage resurfacing procedures for osteochondritis of the knee: A case series. *Am J Sports Med* 2008;36:360-368.
5. Mithoefer K, Williams RJ III, Warren RF, et al. The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. *J Bone Joint Surg Am* 2005;87:1911-1920.
6. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;10:199-206.
7. McIlwraith CW, Frisbie DD, Rodkey WG, et al. Evaluation of intraarticular mesenchymal stem cells to augment healing of microfractured chondral defects. *Arthroscopy* 2011;27:1552-1561.
8. Brittberg M, Winalski CS. Evaluation of cartilage injuries and repair. *J Bone Joint Surg Am* 2003;85(suppl 2):58-69.
9. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: Surgical technique and rehabilitation to treat chondral defects. *Clin Orthop Relat Res* 2001;391(suppl): S362-S369.
10. Martin I, Baldomero H, Bocelli-Tyndall C, Passweg J, Saris D, Tyndall A. The survey on cellular and engineered tissue therapies in Europe in 2010. *Tissue Eng Part A* 2012;18:2268-2279.
11. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* 1991;78:55-62.
12. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-317.
13. Marlovits S, Striessnig G, Resinger CT, et al. Definition of pertinent parameters for the evaluation of articular cartilage repair tissue with high-resolution magnetic resonance imaging. *Eur J Radiol* 2004;52:310-319.
14. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician* 2008;11: 343-353.
15. Lee KB, Hui JH, Song IC, Ardany L, Lee EH. Injectable mesenchymal stem cell therapy for large cartilage defects—A porcine model. *Stem Cells* 2007;25:2964-2971.
16. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. *Am J Sports Med* 2010;38:1110-1116.
17. Sato M, Uchida K, Nakajima H, et al. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. *Arthritis Res Ther* 2012;14:R31.
18. Saw KY, Hussin P, Loke SC, et al. Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: An experimental study in a goat model. *Arthroscopy* 2009;25:1391-1400.
19. Agung M, Ochi M, Yanada S, et al. Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. *Knee Surg Sports Traumatol Arthrosc* 2006;14:1307-1314.
20. Koga H, Shimaya M, Muneta T, et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Res Ther* 2008;10:R84.
21. Sterett WI, Steadman JR, Huang MJ, Matheny LM, Briggs KK. Chondral resurfacing and high tibial osteotomy in the varus knee: Survivorship analysis. *Am J Sports Med* 2010;38:1420-1424.
22. Miller BS, Joseph TA, Barry EM, Rich VJ, Sterett WI. Patient satisfaction after medial opening high tibial osteotomy and microfracture. *J Knee Surg* 2007;20:129-133.
23. Krych AJ, Harnly HW, Rodeo SA, Williams RJ III. Activity levels are higher after osteochondral autograft transfer mosaicplasty than after microfracture for articular cartilage defects of the knee: A retrospective comparative study. *J Bone Joint Surg Am* 2012;94:971-978.
24. Carey JL. Fibrocartilage following microfracture is not as robust as native articular cartilage: Commentary on an article by Aaron J. Krych, MD, et al.: "Activity levels are higher after osteochondral autograft transfer mosaicplasty than after microfracture for articular cartilage defects of the knee. A retrospective comparative study." *J Bone Joint Surg Am* 2012;94:e80.
25. Kobayashi T, Ochi M, Yanada S, et al. A novel cell delivery system using magnetically labeled mesenchymal stem cells and an external magnetic device for clinical cartilage repair. *Arthroscopy* 2008;24:69-76.
26. Kobayashi T, Ochi M, Yanada S, et al. Augmentation of degenerated human cartilage in vitro using magnetically labeled mesenchymal stem cells and an external magnetic device. *Arthroscopy* 2009;25:1435-1441.
27. Hori J, Deie M, Kobayashi T, Yasunaga Y, Kawamata S, Ochi M. Articular cartilage repair using an intra-articular magnet and synovium-derived cells. *J Orthop Res* 2011;29:531-538.