Treatment of Knee Osteoarthritis with Autologous Mesenchymal Stem Cells: A Pilot Study

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Background. Osteoarthritis is the most prevalent joint disease and a frequent cause of joint pain, functional loss, and disability. Osteoarthritis often becomes chronic, and conventional treatments have demonstrated only modest clinical benefits without lesion reversal. Cell-based therapies have shown encouraging results in both animal studies and a few human case reports. We designed a pilot study to assess the feasibility and safety of osteoarthritis treatment with mesenchymal stromal cells (MSCs) in humans and to obtain early efficacy information for this treatment.

Methods. Twelve patients with chronic knee pain unresponsive to conservative treatments and radiologic evidence of osteoarthritis were treated with autologous expanded bone marrow MSCs by intra-articular injection (40×10^6 cells). Clinical outcomes were followed for 1 year and included evaluations of pain, disability, and quality of life. Articular cartilage quality was assessed by quantitative magnetic resonance imaging T2 mapping.

Results. Feasibility and safety were confirmed, and strong indications of clinical efficacy were identified. Patients exhibited rapid and progressive improvement of algofunctional indices that approached 65% to 78% by 1 year. This outcome compares favorably with the results of conventional treatments. Additionally, quantification of cartilage quality by T2 relaxation measurements demonstrated a highly significant decrease of poor cartilage areas (on average, 27%), with improvement of cartilage quality in 11 of the 12 patients.

Conclusions. MSC therapy may be a valid alternative treatment for chronic knee osteoarthritis. The intervention is simple, does not require hospitalization or surgery, provides pain relief, and significantly improves cartilage quality.

Keywords: Osteoarthritis, Articular cartilage, T2 mapping, Mesenchymal stem cells, Stem cell therapy, Regenerative medicine.

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Osteoarthritis is the most prevalent chronic joint disease and a frequent cause of joint pain, loss of function, and disability (*1*). In men ages more than 50 years, osteoarthritis represents the second leading cause of work disability. Furthermore, osteoarthritis is responsible for approximately 2% of all public health expenses (*2*) and large indirect costs derived from productivity decreases (*3*). Many treatments have been proposed but resulted in poor clinical results without cartilage repair (*4*). Articular replacement with prostheses is only recommended as the last treatment option. The American

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J.G.-S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. L.O., R.S., A.S., and J.G.-S. participated in the conception and design of this study. L.O., R.S., and F.S. were primarily responsible for the clinical work. A.M. was responsible for the clinical research and documentation. M.A. and A.S. were responsible for the cell production. M.H. was responsible for the MRI. J.S. was responsible for the statistical analysis. All authors participated in the analysis, discussion, and interpretation of data, contributed to the revision of the article, and gave final approval of the version to be published. J.G.-S. organized all data, conducted meta-analysis and image analysis, and wrote the final draft of the article.

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Academy of Orthopaedic Surgeons recommends only physical and educational therapy, symptomatic treatment with acetaminophen or nonsteroidal anti-inflammatory drugs, and sometimes local corticosteroid injection (*5*). Recommendations of the American College of Rheumatology are very similar (*6*). Common treatments (*7*), including physical therapy (*8*), viscosupplementation (*9*), glucosamine and/or chondroitin sulfate (*10*), arthroscopic surgery (*11, 12*), acupuncture (*13, 14*), and ultrasound (*15*), have demonstrated modest to no clinical benefit compared with placebo.

Cell therapy by surgically implanting autologous chondrocytes has been used to regenerate local cartilage defects for more than 20 years (*16, 17*). Mesenchymal stromal cells (MSCs) have chondrogenic potential (*18, 19*), which is enhanced by coculture with chondrocytes (*20*). Additionally, cocultured MSCs induce chondrocyte proliferation and extracellular matrix protein synthesis, including aggrecan and type II collagen (*21–23*). Therefore, MSCs might be used in place of chondrocytes for cartilage regeneration, and such replacement could be advantageous, especially for diffuse chondral lesions, because MSCs are easier to obtain and expand in vitro without differentiation (*24*). Beneficial MSC effects for chondrogenic repair have been documented in rabbits (*18*), rats (*25, 26*), pigs (*27*), and guinea pigs (*28*). Labeled MSCs injected into the knee joint are still present in the cartilage 1 week after transplantation and migrate, differentiate, and proliferate (*28*). In a recent report, a significant fraction of human MSCs that were injected into rat joints remained 2 to 8 weeks after transplantation. These cells became activated and expressed several human genes that triggered the paracrine expression of collagen II and other chondrogenic rat genes in recipient chondrocytes and resulted in meniscal repair (*26*). Our team performed a feasibility and safety study in three horses; knee joint-injected autologous MSCs were not associated with any identifiable local or general pathologic alterations in necropsy after 6 months. Similar results were obtained in an ovine model (see **Figure S1**, **SDC**, http://links.lww.com/TP/A811).

Cartilage defect repair has been performed in a few human cases by surgically implanting MSCs embedded in collagen pads covered with periosteum (*24, 29*). Autologous MSCs have also been administered by intra-articular injection in two case series with satisfactory results (*30, 31*).

We conducted a pilot study to test the technique's feasibility and safety and to obtain an early indication of the therapeutic value of MSC treatment in 12 human patients with grades II to IV chronic knee osteoarthritis that was unresponsive to conventional treatments. Using autologous bone marrow Good Manufacturing Practice (GMP)–compliant MSCs (*32*) maximized the biosecurity of the protocol based on their extensive use for bone marrow transplantation. The minimally invasive intervention does not require surgery. Our results suggest that MSC treatment improves pain and other clinical signs and, in some cases, delays or even reverts the cartilage damage of osteoarthritis.

RESULTS

Patient Treatment

This study included 12 patients (6 male and 6 female) ages 49±5 years (mean±SE) who were diagnosed with right (n=6) or left (n=6) Kellgren and Lawrence grades II to IV knee osteoarthritis (*33*) by two independent observers. All the selected patients had been unresponsive to conservative treatment (physical and medical) for at least 6 months and nine of them had undergone previous surgery (for more details on antecedent history, see **Table S1**, **SDC**, http://links.lww.com/TP/A811). Patients were recruited between August 2010 and January 2011 and were treated between September 2010 and February 2011. No serious adverse events occurred. Minor adverse events are summarized in Table S2 (see **SDC**, http://links.lww.com/TP/A811). Transient mild local pain and discomfort in the injected knee during the first 1 to 6 days occurred frequently (50% of patients) and was controlled with ibuprofen.

Cell Expansion

The following cell parameters were used (mean±SD; n=12): bone marrow volume, 86±9 mL; number of mononuclear cells obtained, $1.13\pm0.21\times10^9$; expansion time, 22±1 days; number of MSCs, $40\pm1\times10^6$ suspended in Ringer-lactate at 5×10^6 cells/mL; and viability, 91%±6%. Higher cell densities resulted in decreased viability. After 7 to 10 days in culture, cells became relatively homogeneous and demonstrated a fibroblastic appearance when approaching confluence. This morphology remained unchanged until use (*32*). The antigenic profile conformed to the International Society for Cellular Therapy criteria for MSCs (*34*) (see **Figure S2**, **SDC**, http://links.lww.com/TP/A811).

Evolution of Pain, Disability, and Quality of Life

Table 1 summarizes the distribution of knee pain and disability indexes throughout the observation period. The starting point was quite homogeneous in the cohort, with mean values of 45 and 47 for the Visual Analogue Scale (VAS) and Lequesne indexes, respectively. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) values were lower, with pain dominating over rigidity and function loss. These results were consistent with the results obtained in the quality-of-life test (Short Form [SF]-36), where the overall effect was moderate.

Pain was significantly reduced by 3 months after MSC transplantation followed by a smaller additional progressive improvement during the subsequent 9 months (Fig. 1A). Compared with the basal pain level, improvement was statistically significant at all time points. The MSC healing effect was quite rapid: the improvement at 3 months was 69% of the value obtained at 12 months (Fig. 1). The pattern of 1-year improvement was parallel for VAS, WOMAC, and Lequesne indices and resulted in the displacement of the whole distribution toward smaller values, with a strong decrease of median values (P50%) (Table 1). Pain relief during sports performance, followed systematically in eight patients, was even greater and faster (80% at 3 months) (see **Figure S3**, **SDC**, http://links.lww.com/TP/A811). All patients were satisfied with the treatment, and 11 of the 12 patients reported lasting pain relief throughout the 1-year observation period.

Figure 1B shows knee pain relief at the 1-year follow-up, assessed by VAS, as a function of the initial pain score (*35*). Treatment efficacy is equal to the slope of the line, with a slope of 1 (dotted line) indicating the "perfect treatment". An excellent positive correlation was observed between the

TABLE 1. Total score sum of VAS, WOMAC, and Lequesne severity indices

Test	Time	n	Mean	SE	Min	P25%[a]	P50%[a]	P75%[a]	Max
Knee pain VAS-DA (0–100)	0	12	46.9	7.5	0.0	35.8	52.5	66.5	80.0
	3 months	12	25.1	6.8	0.0	3.8	22.5	36.0	74.0
	6 months	12	24.8	6.0	0.0	8.8	16.5	44.0	58.0
	12 months	12	15.4	3.8	0.0	3.0	19.0	24.3	38.0
Knee pain VAS-SP (0–100)	0	8	79.8	6.4	49.0	74.0	88.0	94.5	99.0
	3 months	8	16.4	5.8	1.0	6.0	12.0	21.0	48.0
	6 months	8	11.1	5.1	0	0.0	12.0	26.0	75.0
	12 months	8	15.5	6.4	0	2.3	12.5	31.3	53.0
WOMAC (0–100)									
Pain Subscale	0	12	24.2	4.1	10.0	15.0	17.5	30.0	60.0
	12 months	12	5.8	1.6	0.0	0.0	5.0	10.0	15.0
Rigidity Subscale	0	12	10.4	3.7	0.0	0.0	6.3	15.6	37.5
	12 months	12	5.2	3.2	0.0	0.0	0.0	3.1	37.5
Function loss Subscale	0	12	19.1	3.8	4.4	8.5	14.0	29.0	41.2
	12 months	12	9.4	3.2	0.0	2.2	6.6	12.1	39.7
Total WOMAC Scale	0	12	19.4	3.6	6.3	9.1	14.6	28.1	42.7
	12 months	12	8.3	2.7	0.0	1.8	6.3	12.8	32.3
Lequesne (0–100)	0	12	45.1	5.6	16.7	29.2	43.8	60.4	75.0
	12 months	12	14.9	4.1	0.0	7.3	10.4	21.9	50.0

[a] P25%, P50%, and P75% represent 25th, 50th (median), and 75th percentiles, respectively.

In all cases, the scale was from 0 to 100%. Measurements were performed before cell transplantation (0) and 3, 6, and 12 months afterwards.

Max, maximum value; Min, minimum value; VAS, Visual Analogue Scale; VAS-DA, Visual Analogue Scale for pain associated to daily activities; VAS-SP, Visual Analogue Scale for pain associated to sports activities; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

amount of improvement and the initial score (r=0.86), indicating that MSC treatment had a clear pain-relieving effect ($P<0.001$). The slope of the line was 0.69. The evolution of the Lequesne algofunctional index was very similar (Fig. 1C), wherein correlation between improvement and the initial score was good (r=0.70; $P<0.01$). The slope of this line was 0.65. Similar observations were found for the WOMAC index. The pain and physical function loss values are shown in Figure 1D. For the pain component, the correlation between improvement and the initial pain score was also very good (r=0.92; $P<0.001$); the efficacy was 0.78. The other components followed the same trends, but the numerical values were smaller.

The SF-36 Quality of Life Questionnaire revealed a very modest impact of MSC therapy by the end of the follow-up period. The differences between baseline and treated values were not significant for any of the eight test subscales (data not shown). The SF-36 questionnaire is known to be less sensitive for assessing knee arthritis than the WOMAC, which was developed specifically for patients with lower extremity arthritis (*36*). In fact, in several prior studies, the SF-36 scores were scarcely modified in either control or treated osteoarthritis patients (*12, 14*). Thus, we place more value on the WOMAC scoring system.

Imaging

Magnetic resonance imaging (MRI) quantitative T2 mapping was used to evaluate articular cartilage quality (*37, 38*). T2 relaxation time is sensitive to both changes in cartilage hydration and collagen fibril orientation (*39–41*). T2 relaxation time is longer in remodeling inflammatory tissue versus hyaline cartilage (*40–43*) and increases in osteoarthritis (*39, 44, 45*).

Consistent with previous results in the healthy knee (*39–41, 43, 44*), the mean±SD T2 value was 37.0±6.8 ms (see **Figure S4A**, **SDC**, http://links.lww.com/TP/A811). Because 95% of values should be smaller than (mean+2×SD), 50 was chosen as the threshold above which T2 values were considered inordinately high. To quantify T2 mapping, a Poor Cartilage Index (PCI) was estimated as the percentage of T2 values larger than 50 ms. A PCI of 100 is the worst possible value, and a value near 5 is considered healthy. A positive correlation was identified between the baseline PCI and VAS scores (r=0.42; $P<0.001$) (see **Figure S4B**, **SDC**, http://links.lww.com/TP/A811). Additionally, the mean PCI significantly decreased from 19.5 to 15.4 during the first 6 months after treatment and further decreased to 14.3 at 12 months after injection (Fig. 2A). Figure S4C details individual patient evolution (see **SDC**, http://links.lww.com/TP/A811). The PCI decreased in 11 of 12 patients. Additionally, when PCI improvement was plotted against the initial PCI, a positive correlation (r=0.64; $P<0.020$) was noted. The slope of the best-fitting line was 0.27 (Fig. 2B).

DISCUSSION

Both animal experimentation and human case studies suggest that intra-articular MSC injection could be a useful therapeutic alternative for treating knee osteoarthritis. Our preliminary studies in horses and sheep (see **Figure S1**, **SDC**, http://links.lww.com/TP/A811) demonstrated procedural feasibility and safety. Here, we present a phase I/II study of

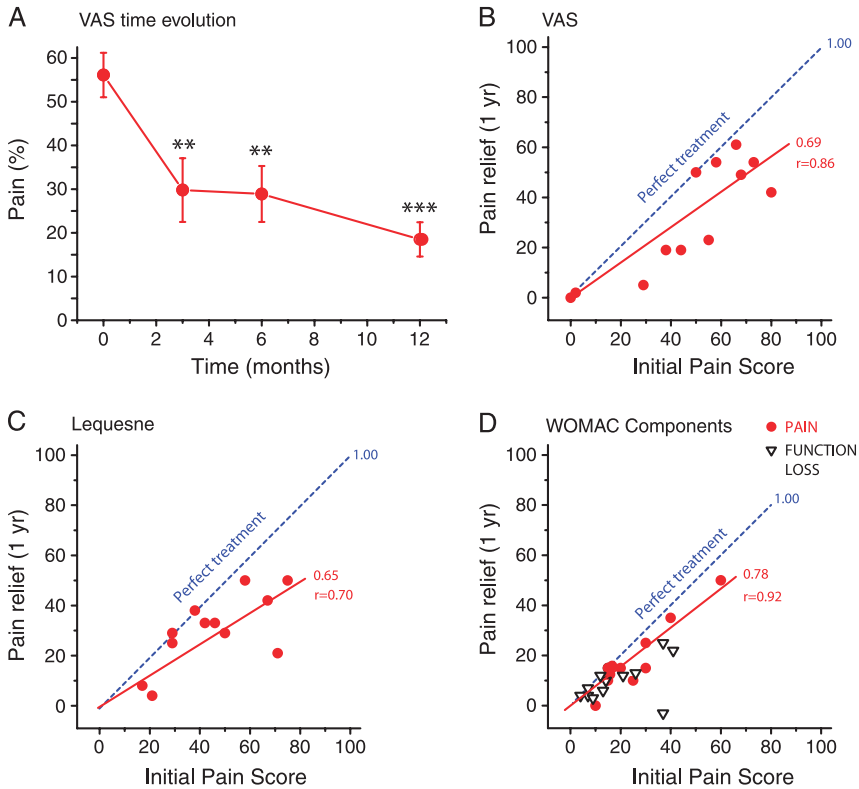

FIGURE 1. Pain improvement resulting from MSC treatment. A, evolution of knee pain over time, as measured by the VAS. Mean±SE values of 12 patients treated with MSCs. $^{**}P<0.01$; $^{***}P<0.001$ (ANOVA; Bonferroni test for paired values). B–D, correlation between improvement of knee pain 1 year after treatment with MSCs and initial pain score, as measured with different tests. The "perfect" treatment (dotted line with slope of 1) is shown for comparison. The best-fitting lines are shown with values for the slope and linear regression coefficient (r) at the right. In case D, the pain and physical function loss subscales of the WOMAC test are shown with different signs (codes at top right). ANOVA, analysis of variance; MSC, mesenchymal stem cells; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

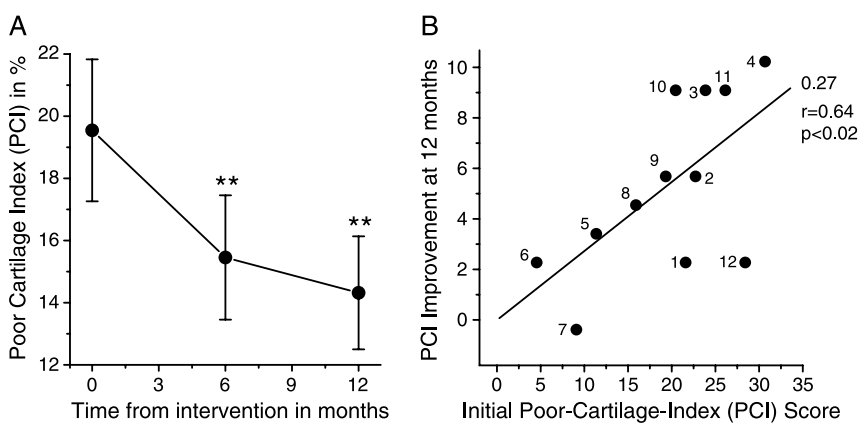

FIGURE 2. Cartilage quality improvement resulting from MSC treatment. Cartilage quality was assessed by MRI T2 mapping and is quantified as the PCI (computed as the percentage of sample points with a T2 relaxation value >50 ms). The worse possible value for PCI is 100, and healthy cartilage should approach 5. A, temporal evolution of PCI. Mean±SE values of 12 patients treated with MSCs. $^{**}P<0.01$ (ANOVA; Bonferroni test for paired values). B, correlation between PCI improvement and initial PCI score for the 12 patients included in this study. Codes for each patient are given beside the data points. The best-fitting line is shown with values for the slope and linear regression coefficient (r) at the right. ANOVA, analysis of variance; PCI, Poor Cartilage Index; MRI, magnetic resonance imaging; MSC, mesenchymal stem cells.

12 patients with clinical and objective follow-up coverage for 1 year after intra-articular MSC injection. Our results show that autologous MSC transplantation is both feasible and safe, with no major adverse events recorded. The postimplantation pain observed in 50% of patients responded well to ibuprofen and vanished within 1 to 6 days. Quality control and reproducibility of cell production is essential for meaningful evaluation of cell therapy trials. The GMP-compliant cell preparation (*32*) was very reproducible with respect to the number of cells (SD=3%) and the expansion time (SD=5%). Immunophenotypic characteristics were also adequate and stable over time. Cell viability was more than 90% and not affected by transport to the administration site.

The analgesic effect of MSC treatment is remarkable, resulting in 65% to 78% improvement in pain (Fig. 1B–D; see **Figure S3**, **SDC**, http://links.lww.com/TP/A811). Improvements in function (Fig. 1D) and quality of life are smaller. Our results supersede those of previous case reports, where results were described as "satisfactory" (*31*) or "encouraging" (*30*). In these case studies, the number of cells used was smaller ($8–20\times10^6$), follow-up was for only 6 months, and the MRI study, when performed, was not quantitative.

Figure 3 presents a meta-analysis of four recent high-quality clinical trials (*8, 11, 12, 14*). Data on pain evolution were recalculated and expressed on a 0 to 100% scale. Quantification and comparison of several osteoarthritis treatments

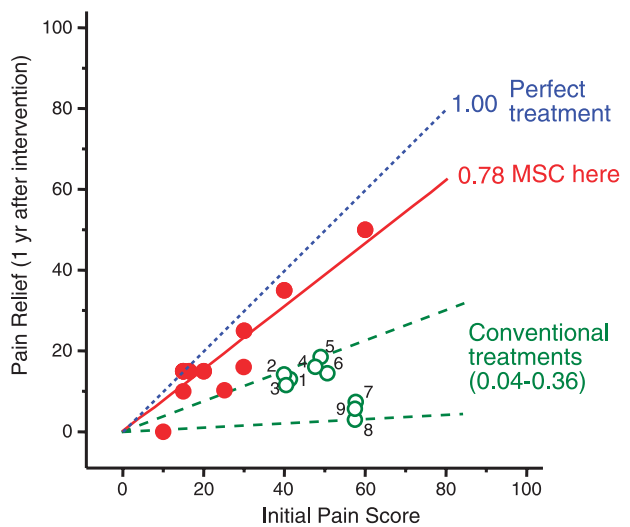

FIGURE 3. Comparison of the efficacy of several osteoarthritis treatments for pain relief. Data from four prestigious clinical trials (*8, 11, 12, 14*), quantified using the algofunctional WOMAC index, are represented as pain relief versus initial pain score (*35*). The slope of the lines (values at right) represents efficacy. Lines were forced to pass through the origin. The data from the present study ("MSC here") are included for comparison (each closed circle corresponds to one patient; three values overlap at 15,15 location). Open circles correspond to results obtained in different trials; the definition of the numerical codes is given in the last column of Table S3 (see **SDC**, http://links.lww.com/TP/A811). For a more detailed description, see **Table S3** (**SDC**, http://links.lww.com/TP/A811). MSC, mesenchymal stem cells; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

were performed using the initial pain score versus pain relief plot (*35*). The slope of the line defines the treatment efficacy, with complete pain relief reflected in a slope of 1. Each point represents a given condition (for codes, see last column in Table S3 [see **SDC**, http://links.lww.com/TP/A811], which also provides additional trial details). Overall, the slopes oscillated between 0.04 and 0.36 (mean±SE, 0.21±0.04) for seven conventional treatments (see **Table S3**, **SDC**, http://links.lww.com/TP/A811). Our results, labeled "MSCs here" in Figure 3, compare very favorably with previous trials that explored conventional treatments (*8, 11, 12, 14*).

The analgesic effect of MSC treatment was quite rapid, with more than 50% of the total improvement attained by 3 months (Fig. 1A). For sports activities-associated pain, the improvement was even faster (see **Figure S3**, **SDC**, http://links.lww.com/TP/A811). Early action has also been reported for the effects of MSCs on degenerative disc disease (*32*). After this rapid effect, improvement progressed more slowly and the maximum effect was observed at the 1-year follow-up. Pain improvement associated with sports activities was even larger than the pain improvement associated with daily activities (Figure S3).

Our novel approach for analyzing T2 mapping images filters out most of the spurious variations and enhances sensitivity by focusing on the evolution of the poor cartilage areas. We demonstrate a significant correlation between the PCI and the VAS (see **Figure S4B**, **SDC**, http://links.lww.com/TP/A811). Additionally, the PCI was improved significantly by MSC treatment (Fig. 2A), although the magnitude of this effect varied among cases (see **Figure S4C**, **SDC**, http://links.lww.com/TP/A811). Finally, the slope of the relationship between PCI improvement and initial the PCI was 0.27 (Fig. 2B), suggesting that cartilage healing, although significant ($P<0.01$), was less than the analgesic effect. Further investigation of cartilage healing progression over longer evolution times, and the effect or repeated MSC application, will be informative.

We can only speculate regarding the mechanisms governing the beneficial effects of MSC treatment. Chondrocytes induce differentiation of cocultured MSCs toward a chondrocyte phenotype (*20*). Proliferation and differentiation of MSCs to chondrocytes also happen with MSCs injected into knee joints (*28*). Importantly, MSCs stimulate cocultured cells to proliferate and synthesize extracellular matrix (*21, 23, 46*). This action may be more important in vivo because few MSCs are required to trigger this effect (*22*). It was recently shown that transplanted MSCs engraft into the joint, are activated, and express Indian hedgehog and other genes. These genes in turn promote expression of collagen II and other chondrogenic genes by host cells (*26*). Additionally, MSCs have a well-known immunomodulatory effect (*47, 48*) and can induce anti-inflammatory cytokine production (*22*). These data indicate that MSCs may help analgesia by reducing inflammation. Because the analgesic effect is more evident than anatomic restoration, we conclude that the trophic and anti-inflammatory effects of MSCs on the damaged tissue may occur more quickly than the regenerative effects.

In summary, we propose that cell therapy with expanded bone marrow–derived MSCs should be considered as a putative treatment for chronic osteoarthritis. Cell handling and expansion is reproducible, and quality-control tests were satisfactory. The clinical procedure is feasible and safe and requires

only minimally invasive intervention without surgery or hospitalization. The results are better than those obtained with established treatments. Pain relief occurs by 3 months and increases for at least 1 year. The recovery of functional losses is less but also significant, and there is quantitative evidence of partial articular cartilage healing. Future studies will involve larger trials focused on efficacy, with greater patient numbers and longer follow-up periods. These studies will track long-term joint evolution and investigate the specific anatomic and functional changes that occur in the knee.

MATERIALS AND METHODS

Patients and Procedures

This pilot phase I/II trial was approved by the Teknon Medical Centre Ethics Committee and the Spanish Drug and Medicines Agency (EudraCT 2009-017405-11) and registered in ClinicalTrials.gov (NCT01183728). Twelve patients with chronic knee osteoarthritis unresponsive to conventional treatments (for details, see **Table S1**, **SDC**, http://links.lww.com/TP/A811) were included. Detailed inclusion and exclusion criteria are reported in Table 2. After clinical, analytical, and imaging evaluations to ensure compliance with these criteria, patients were informed about the protocol characteristics and provided written informed consent.

The protocol included seven visits (V0–V6). V0 involved the final check of compliance with inclusion criteria, performance of necessary complementary evaluations and tests, and scheduling of dates for V1 and V2. V1 involved bone marrow harvesting from the iliac crest (80–90 mL) for MSC isolation. This intervention was performed under local anesthesia and slight sedation, and patients were discharged after 2 hr of observation. V2 (21–24 days after V1) involved the injection of MSCs (40×10^6 cells per knee from a 5×10^6 cells/mL suspension by medial parapatellar injection). V3 to V6 (8 days and 3, 6, and 12 months after implantation) included clinical evaluation and routine analysis (V3–V6), VAS for daily activity and for sports (*35*), WOMAC and Lequesne algofunctional indices (*49*), SF-36 questionnaire (*50*), and quantitative MRI exploration (V0, V5, and V6). Outcomes were expressed on a 0 to 100% scale in all cases.

TABLE 2. Inclusion and exclusion criteria

Inclusion criteria
1. Grade II to IV osteoarthritis according to the Kellgren–Lawrence grading scale (*33*) and concurred by two different observers.
2. Chronic knee pain of mechanical origin.
3. Absence of local or general infection.
4. Hematologic and biochemical analyses with no significant alterations that contraindicate intervention.
5. Patient is able to understand the nature of the study.
6. Informed written consent provided by the patient.
Exclusion criteria
1. Age >75 or <18 years or legally dependent.
2. Signs of infection or positive serology for HIV, hepatitis, or syphilis.
3. Congenital or acquired diseases leading to significant knee deformities that may interfere with cell application or inter pretation of results.
4. Obesity, with body mass index >30 (calculated as mass in kg/height in m^2).
5. Pregnancy or breast-feeding.
6. Neoplasia.
7. Immunosuppression.
8. Intra-articular injection of any drug during the previous 3 months.
9. Participation in another clinical trial or treatment with an other investigational product within 30 days before inclusion in the study.
10. Other conditions that may, according to medical criteria, discourage participation in the study.

Cell Isolation and Expansion

Cell isolation and expansion were performed in the Instituto de Biología y Genética Molecular Cell Production Unit under GMP conditions and with approval of the Spanish Drug and Medicines Agency (PEI No. 10-134), as described previously (*32*). Bone marrow samples were transported to the Cell Production Unit at 4°C to 12°C within 12 hr of harvesting. The mononuclear cell fraction was isolated by density-gradient centrifugation, resuspended, and cultured in MSC expansion culture medium (*51*) in 175-cm^2 tissue culture flasks, with periodic washing to remove nonadherent cells. When cells reached 80% confluence, they were trypsinized and replated, and the process was repeated for two more passages. At the end of this period (21–24 days), cells were harvested, resuspended in Ringer's lactate solution containing 0.5% human albumin (CSL Behring GmbH, Marburg, Germany) and 5 mM glucose, and transported at 4°C to 20°C by air courier (6 hr) to Teknon Medical Centre for application. In addition to quality-control tests, viability and flow cytometric immunophenotypic profiles (*34, 51*) were determined at this stage.

MRI Assessments

MRI was used to assess cartilage state by T2 mapping using the GE CartiGram sequence (*37–39*). Mean T2 relaxation values (ms) were sampled in 88 well-defined regions of interest (ROIs), including patellar cartilage (24 ROIs), femoral condyles (32 ROIs), and tibial condyles (32 ROIs). Instrumental variation, computed as the mean of differences between two consecutive measurements, was approximately 4%. Interobserver variation was 3%. To analyze assay results, values were averaged in each area and those above 50 ms, which represent poor quality, remodeling, inflammatory tissue (*40–42*), were counted to compute the PCI (expressed as percentage of all values obtained in the 88 ROIs) as described in Results. Values above 90 were not used for computations. For the PCI, 100% represents the worst possible PCI value and values at or below 5% are considered healthy.

Statistical Analysis

Data are reported as mean±SD (or mean±SE), as indicated. The significance of differences was assessed either by Student's *t* test or by one-way analysis of variance (ANOVA) and the corresponding nonparametric tests. GraphPad Instat3 package software version 3.06 (GraphPad Software, La Jolla, CA) was used for calculations

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