Treatment of Knee Osteoarthritis With Allogeneic Bone Marrow Mesenchymal Stem Cells: A Randomized Controlled Trial

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Background. Osteoarthritis is the most prevalent joint disease and a common cause of joint pain, functional loss, and disability. Conventional treatments demonstrate only modest clinical benefits without lesion reversal. Autologous mesenchymal stromal cell (MSC) treatments have shown feasibility, safety, and strong indications for clinical efficacy. We performed a randomized, active control trial to assess the feasibility and safety of treating osteoarthritis with allogeneic MSCs, and we obtain information regarding the efficacy of this treatment. **Methods.** We randomized 30 patients with chronic knee pain unresponsive to conservative treatments and showing radiological evidence of osteoarthritis into 2 groups of 15 patients. The test group was treated with allogeneic bone marrow MSCs by intra-articular injection of 40×10^6 cells. The control group received intra-articular hyaluronic acid (60 mg, single dose). 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 indications of clinical efficacy were identified. The MSC-treated patients displayed significant improvement in algofunctional indices versus the active controls treated with hyaluronic acid. Quantification of cartilage quality by T2 relaxation measurements showed a significant decrease in poor cartilage areas, with cartilage quality improvements in MSC-treated patients. **Conclusions.** Allogeneic MSC therapy may be a valid alternative for the treatment of chronic knee osteoarthritis that is more logistically convenient than autologous MSC treatment. The intervention is simple, does not require surgery, provides pain relief, and significantly improves cartilage quality.

(*Transplantation* 2015;99: 1681–1690)

Osteoarthritis is the most prevalent chronic joint disease and a frequent cause of joint pain and disability.[1] Unfortunately, conventional treatments demonstrate only modest clinical benefits[2,3] and articular replacement by prosthesis is recommended only as a last treatment option. Cell-based therapies are promising for the treatment of osteoarthritis and have shown encouraging results in animal studies and in human case reports. In fact, we recently published the results of a phase I/II pilot clinical trial for knee osteoarthritis with autologous bone marrow (BM)-derived mesenchymal stromal cells (MSC). We confirmed the feasibility and safety of MSC treatments, and our results provided strong indications for clinical efficacy. Patients exhibited rapid and progressive improvement of algofunctional indices, which approached 65% to 78% efficiency within a year. The outcomes were better than those of conventional

Received 15 July 2014. Revision requested 22 November 2014.

Accepted 27 November 2014.

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Financial support was provided by the Program for Support of Independent Clinical Research of the Spanish Ministerio de Sanidad (EC11-309), *Red de Terapia Celular* (RD06/0010/0000 and RD12/0019/0036) of the Instituto de Salud Carlos III, Ministerio de Economia y Competitividad, and the *Centro en Red de Medicina Regenerativa de Castilla y León*.

The authors declare no conflicts of interest.

Clinical trial registries: EudraCT 2011-005321-51, ClinicalTrials.gov: NCT01586312

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

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Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

ISSN: 0041-1337/15/9908-1681

DOI: 10.1097/TP.0000000000000678

treatments,[4] and the associated improvements were maintained at the 2-year follow-up.[5] A recent study with MSCs from a different origin, autologous adipose tissue-derived MSC, has confirmed significant clinical benefit 6 months after treatment.[6] In all MSC studies, objective proof of cartilage improvement by magnetic resonance imaging (MRI), arthroscopic examination, and histological analysis of biopsy specimens was provided.[4-6]

Autologous MSCs are an excellent therapeutic option for treating osteoarthritis; however, cell expansion makes the procedure slow and expensive. Allogeneic cells would be cheaper and, logistically, more convenient. The most obvious disadvantage of using these cells is the possibility of host immune rejection. Mesenchymal stromal cells, however, are *immune privileged*[7] or *immune evasive*[8] and inhibit immune responses in a manner not restricted by the HLA system. As a result, nonmatched MSC are better tolerated than other cell types. In fact, there are no reports of rejection in animal experiments,[9-12] and studies of transplanted MSC persistence show the same values results for autologous and allogeneic cells.[8,13] In humans, excellent tolerance to allogeneic MSCs has been reported in many clinical trials. For example, in a recent meta-analysis of 87 lupus erythematosus patients, no transplantation-related adverse events were found after 4 years of follow-up.[14] Similarly, no transplantation-related adverse events occurred in MSC-treated patients with breast cancer,[15] left ventricular dysfunction,[16] ankylosing spondylitis,[17] graft versus host disease,[18] and other autoimmune diseases.[19] Additionally, in the osteoarticular field, a recent meta-analysis of 844 intra-articular transplantations of allogeneic MSC concluded that the procedure is safe after a 21-month follow-up.[20]

We present a randomized controlled comparator multicenter study to assess the feasibility and safety of using allogeneic MSCs to treat knee osteoarthritis. We present evidence to suggest that intra-articular injection of allogeneic MSCs has therapeutic value for treating knee osteoarthritis. Bone marrow good manufacturing practice–compliant MSCs[21] are safe and have been used extensively for BM transplantation. For this reason, we selected these cells to maximize biosafety and efficacy. We used an established hyaluronic acid treatment[22] as the active control. The intervention used to introduce MSCs into the joint was minimally invasive and did not require surgery, which reduced associated complications. Our results suggest that allogeneic MSC treatment improves both pain and cartilage quality and may be a valid alternative (cheaper than autologous MSC) for treatment of chronic knee osteoarthritis.

MATERIALS AND METHODS

Patients and Procedures

This multicenter phase I-II trial was approved by Ethics Committees at Valladolid University Hospital and the Teknon Medical Center and by the Spanish Agency of Medicines (EudraCT 2011-005321-51). The study was registered at ClinicalTrials.gov (NCT01586312). The design of this trial was based on our previous one, performed with autologous MSC.[4] Sample size was determined based on the results from the previous trial for α risk = 0.05 and β risk = 0.20. We recruited 30 patients (13 men and 17 women; mean age ± SE = 57 ± 9 years) with grade II-IV[23] chronic knee osteoarthritis (**Figure S1, SDC,** http://links.lww.com/TP/B133), and unresponsive to conventional treatments (physical and medical) for at least 6 months before recruitment. Recruitment was performed between April and September 2012. Detailed inclusion and exclusion criteria are reported in Table 1. For details on antecedent patient history, see Table 2. The recruited patients were block randomized by the quality control manager of the cell production facility, who was blinded, to receive either the control or the experimental treatment. The allocation ratio was 1:1. All patients were treated between July and December 2012, either in the Valladolid University Hospital (26 patients) or at the Barcelona Teknon Medical Center (4 patients). Clinical, analytical, and imaging evaluations were performed to ensure compliance with these criteria. Patients were informed of the protocol design before providing written informed consent. The protocol included 6 visits (V0-V5). The V0 visit involved a final compliance check using the inclusion criteria, performance of necessary complementary evaluations and tests, and scheduling of dates for the next visit. At V1, treatments were administered, either MSCs (40×10^6 cells/knee from a 5×10^6 cell/mL suspension by medial parapatellar injection) or hyaluronic acid (60 mg in 3 mL; Durolane) were injected. The V2 to V5 visits (8 days, and 3, 6, and 12 months after implantation) included clinical evaluation and routine analyses (V2-V5) using the visual analogue scale (VAS),[24] Western Ontario and McMaster Universities Osteoarthritis (WOMAC),[25] and Lequesne algofunctional indices,[26] as well as a short form-12 life quality (SF-12) questionnaire[27] and quantitative MRI exploration

TABLE 1.

Inclusion and exclusion criteria

Inclusion criteria
1. Grade II-IV osteoarthritis, identified by two different observers, according to the Kellgren-Lawrence grading scale[23]
2. Chronic knee pain of mechanical origin
3. Absence of local or general infection
4. Hematological 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 the interpretation of results
4. Obesity with a 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 another investigational product within 30 days prior to inclusion in the study.
10. Other conditions that may, according to medical criteria, discourage participation in the study

HIV, human immunodeficiency virus

TABLE 2.

Antecedent history of the patients included in this trial

ARM	Patient No.[a]	Sex	Age	Side	OA Grade	Previous surgery (date)	Cortic. N (date)	HA N (date)	PRP N (date)
Control (+HA)	1	F	71	R	IV				
	2	F	52	R	II			3 (2011), 2 (2012)	
	6	M	51	R	III				
	8	F	56	R	II	MM (2006)			
	9	F	63	R	II				
	11	M	66	R	II		2 (2011)		
	14	M	51	R	II	(R) Chondropathy (2010)			
	17	F	59	R	III	MM (1981)			
	18	F	44	L	II	MM (2002-2012)		3 (2012)	
	21	F	63	R	III	MM (2002)(2004), MM (L)(1998)		3 (2011), 3 L (2011)	
	22	F	64	R	IV	(R) Patellar fracture (1997)			
	23	M	68	L	IV		1 (2012)		
	24	M	56	R	III	MM (2010)		3 (2011)	
	27	F	36	R	II	LM (1988), LM (1990), ACL (2000), RT (2000)	3 (2009)	1 (2010)	4 (2011)
	29	F	60	L	III	LM (1982)			4 (2011)
ARM	Patient No.	Sex	Age	Side	OA Grade	Previous surgery (date)	Cortic. N (date)	HA N (date)	PRP N (date)
TEST (+MSC)	3	F	64	L	III	MM (2008)			
	4	M	65	L	III				
	5	M	56	R	IV				
	7	F	38	R	II	MM (1999), LM (2001)			
	10	M	60	R	II	Arthroscopic surgery (L) (3 times in 2009)			
	12	M	52	L	III	MM (R)(1986). MM (1990)			
	13	F	50	R	II			5 (2012)	
	15	M	52	R	IV	MM (2006)		4 (2010)	
	16	F	42	R	II	MM (2005)			
	19	F	64	R	III	MM (1998)			
	20	F	73	R	III	MM (1994), MM (L)(1995)		1 (1998), 1 (2011)	3 (2012)
	25	F	53	L	II				
	26	M	52	R	II	MM (1981)			
	28	F	68	L	III		1 (2011)	1 (2005)	4 (2010)
	30	F	60	R	IV	LM (1970), ACL (1970)			4 (2011)

Date (year) given in brackets. L and R, left and right, given in brackets in "Surgery". When not indicated, side is the same as the lesion. All the patients received rehabilitation and a non-steroidal antiinflammatory drugs.

[a] Cells from donor 1 were used for treatment of patients 3, 4, 5, and 7; cells from donor 2 for patients 10, 12, 13, 15, and 16; and cells from donor 3 for patients 19, 20, 25, 26, 28, and 30.

OA, osteoarthritis; Cortic., infiltration with corticosteroids; HA, hyaluronic acid; PRP, platelet-rich plasma; (N), number of applications; ACL, anterior cruciate ligament; MM, medial meniscus; LM, lateral meniscus; MCL, medial collateral ligament; QRT, quadriceps retensioning.

(V0, V4, and V5). Outcomes were expressed using a 0% to 100% scale in all cases. The patients, radiologists, care providers, and persons assessing the outcomes of the assay were blinded after the assignment.

Cell Isolation and Expansion

Bone marrow mesenchymal stromal cells, were obtained from 3 healthy donors that were subjected to autologous MSC transplantation and produced more cells than needed for their own treatment. Cells were processed using good manufacturing practice conditions in the IBGM Cell Production Unit as described previously.[4,21] Isolations were carried out with the following parameters (mean ± SD; n = 3): BM volume = 103 ± 8 mL, number of mononuclear cells obtained = $1.1 \pm 0.5 \times 10^9$, expansion time = 22 ± 2 days, number of MSC injected into the knee = 40×10^6 while suspended in Ringer-lactate at 5×10^6 cells/mL, and viability greater than 98%. A serum sample from each donor was obtained to screen for human immunodeficiency, hepatitis B, and hepatitis C virus by Nucleic Acid Amplification Technology.[28] The cells obtained from each donor were used for 4 to 6 recipients (see Table 2 legend for details). Immune matching was not attempted.

Bone Marrow Harvesting, Purification, and Culture

Puncture and BM aspiration were performed in an ambulatory surgery session. The patient, in prone position, underwent light sedation. The surgical field was brushed with alcoholic povidone-iodine solution (chlorhexidine if a history of allergy to iodine exists) and delimited with sterile fields, leaving free both posterior iliac crests. After local anesthesia (20 mL of 1% lidocaine without epinephrine diluted v/v with saline), 2 members of the extractor team, placed on both sides of the operating table, performed several punctures with an 11-G trocar under

the iliac spine, aiming toward the posterior sacroiliac joint (this is the iliac area with higher trabecular density). The technique involves sudden cortical perforation and repeated aspiration of small BM volumes (2-4 mL) to minimize contamination with the peripheral blood. The aspirate was injected into a heparinized bag for transport. Two successive aspirations were performed by rotating 90 degrees clockwise the beveled trocar. The same puncture hole allows a further 1 to 2 mm deepening twice, repeating the same methodology with 2 to 4 mL suction, syringe change, 90 degrees bezel rotation, and new aspiration. Then the trocar can be removed by sliding it slightly, and a few millimeters above the cortical pelvic, puncture can be repeated, continuing on both sides of the pelvis until about 80 mL are collected. Bone marrow (sterile bag heparinized with a volume of about 80 mL of aspirate) was refrigerated to 4°C, conditioned, and shipped to the Cell Therapy Unit. Further processing was done within 24 hours.

Donor serum samples were also obtained at this time to carry out the required screenings for excluding human immunodeficiency virus and hepatitis A and B contamination (Annex VIII and Directive 2004/23/EC of the European Parliament and of the Council). Tests must be performed by Nucleic Acid Amplification Testing to circumvent quarantine, which would otherwise be necessary to avoid the window effect.[28] An aliquot of the serum sample must be stored to allow further analysis if required in future.

Bone marrow samples were transported to the cell production unit at 4 to 12°C within 24 hours of harvesting. The mononuclear cell fraction was isolated by density-gradient centrifugation, resuspended, and cultured in MSC expansion culture medium[29] 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 2 more passages. At the end of this period (21-24 days), cells were harvested, resuspended in Ringer lactate solution containing 0.5% human albumin (CSL Behring GmbH, Marburg, Germany) and 5 mM glucose, and transported at 4 to 20°C to the hospital application. In addition to quality-control tests, viability and flow cytometric immunophenotypic profiles[29,30] were determined at this stage.

MRI Assessments

Magnetic resonance imaging was used to assess the cartilage state by T2 mapping using the GE CartiGram as described previously[4] at baseline and 6 and 12 months after treatment. Briefly, mean T2 relaxation values (ms) were sampled in 88 well-defined regions of interest (ROIs) including at the patellar cartilage (24 ROIs), femoral condyles (32 ROIs), and tibial condyles (32 ROIs). We have shown previously that values in healthy individuals distribute normally, with a mean (and median) of 37 ms and a SD of 7 ms. The 95% percentile value was 50 ms.[4] To analyze the assay results, we averaged the values in each area (88 ROIs). Those above 50 ms, which reveal poor-quality remodeling inflammatory tissue,[31-33] were counted to compute the poor cartilage index (PCI, expressed as a % of all values obtained in the 88 ROIs), as described in the Results. Values above 90 ms were not used for computations. Values at 100% represent the worst possible PCI, and those at 5% or below are considered healthy. The MRI tests were completed in all the 15 control patients and 12 of the 15 test patients.

Statistical Analyses

Data are reported as means ± SD or SE, as indicated. Significant differences were assessed by either Student *t* tests or by 1-way repeated-measures analyses of variance with appropriate corresponding nonparametric tests. We used GraphPad Instat3 package software version 3.06 (GraphPad Software, La Jolla, CA) for all calculations.

RESULTS

Patient Treatment

The 30 patients recruited for this study were given randomized treatments of either MSCs or hyaluronic acid. No serious adverse events occurred during treatment. Minor adverse events are summarized in Table 3. Transient mild local pain and discomfort in the injected knee with inflammation and swelling during the first 1 to 7 days occurred frequently in both the control and cell-treated patients (50-60% of patients). This pain was controlled with ibuprofen.

Cell Expansion

Cells obtained from the 3 healthy donors were relatively homogeneous and demonstrated a fibroblastic appearance when approaching confluence after 7 to 10 days in culture. This morphology remained unchanged until use. The antigenic

TABLE 3.

Minor adverse events

Minor adverse event (comments)	Controls (%)	MSV-treated (%)	Overall (%)
Postimplantation pain and/or synovial fluid effusion with articular swelling at days 1-7 (E, SR). Responds to ibuprofen. Endurance < 1 week.	9 (60%)	8 (53%)	17 (57%)
Unexpected osteoarticular pain and/or inflammation (knee, shoulder, hip, ankle, lumbar) (NSR, UE)	5 (33%)	7 (47%)	12 (40%)
Other: Menstrual disorders, influenza, migraine, toothache, restlessness, memory loss, testicular pain, rhinitis, sensitive hand alteration, sleepiness, allergic reaction, tinnitus, dental implant, lipoma, skin tumour) (NSR, UE)	11 (73%)	8 (53%)	19 (63%)

The total number of patients was 30, 15 in each arm.
In all cases, the adverse events responded to medical/physical therapy.
Comments: E, expected; UE, unexpected; SR, study-related; NSR, not study-related.

TABLE 4.

Total scores for the VAS, WOMAC, and LEQUESNE severity indices

Test	C or E	Time	N	Mean	SE	Min	P25%[a]	P50[a]	P75[a]	Max	Conf 95[a]	Conf 99[a]
VAS (0-100)	C	Baseline	15	64	7	4	54	70	79	97	13	17
	C	12 mo	15	51	8	1	27	50	73	98	15	20
			SMD[b] = 0.48									
	E	Baseline	15	54	7	15	49	55	70	81	10	13
	E	12 mo	15	33	6	1	13	27	50	82	13	17
			SMD[b] = 0.77									
WOMAC-pain (0-100)	C	Baseline	15	50	4	20	47	51	57	71	7	10
	C	12 mo	15	44	6	7	25	44	55	86	12	16
			SMD[b] = 0.39									
	E	Baseline	15	46	4	24	35	43	59	70	8	10
	E	12 mo	15	30	4	8	21	28	36	75	8	10
			SMD[b] = 1.03									
WOMAC-general (0-100)	C	Baseline	15	45	3	18	35	46	56	65	6	8
	C	12 mo	15	41	6	6	24	33	56	86	12	15
			SMD[b] = 0.34									
	E	Baseline	15	41	3	17	31	44	51	64	7	10
	E	12 mo	15	28	5	6	17	23	35	78	9	12
			SMD[b] = 1.12									
LEQUESNE (0-100)	C	Baseline	15	45	4	13	35	46	56	63	7	9
	C	12 mo	15	42	5	13	29	38	56	75	9	12
			SMD[b] = 0.19									
	E	Baseline	15	39	4	17	38	38	44	67	7	9
	E	12 mo	15	30	3	13	17	29	40	50	6	8
			SMD[b] = 0.58									

[a] P25%, P50%, and P75% represent 25th, 50th (median), and 75th percentiles, respectively.
[b] SMD = standardized mean difference, computed as improvement (baseline value minus value at the end of treatment) divided by the SD of the baseline value. SMD is used here as an estimate of effect size. The correlation between effect size and magnitude of the change are: 0 = null, 0.20 = small, 0.50 = medium, 0.8 = large. Details on effect size can be found in Kirkley et al.[34]
In all cases, the scale was from 0% to 100%. Measurements performed before cell transplantation (baseline) and 12 months afterwards are shown.
Min, minimum value; Max, maximum value; Conf 95 and Conf 99, confidence interval at 95 and 99% significance.
C, control patients (hyaluronic acid); E, experimental cell-treated patients

profile of the cultures conformed to the International Society for Cellular Therapy criteria for MSCs.[4,21,29,30]

Evolution of Pain, Disability, and Quality of Life

Table 4 summarizes the distribution of knee pain and disability indices throughout the observation period. The starting point within the cohort ranged from mean values of 39 and 64 on a scale of 0 to 100 for the VAS, Lequesne index, and WOMAC index. Values of all evaluation scales improved more with the cell treatment than with the active control (hyaluronic acid). Improvement of patients following cell treatment was medium to large (effect size, 0.58 to 1.12 for the different algofunctional indices, Table 4), whereas improvement was small (effect size, 0.19 to 0.48) after hyaluronic acid treatment.

Pain was significantly reduced by 6 and 12 months after MSC transplantation (VAS and pain subscale of WOMAC, Figure 1A and B). The active controls treated with hyaluronic acid showed smaller improvements, not statistically significant except for the VAS value12 months after intervention (Figure 1A and B). The other algofunctional indices (general scale of WOMAC and Lequesne) changed consistently, with significant decreases at 6 and 12 months in the MSC-treated group and no significant change in the active controls (Figure 1C and D). The observed changes were parallel for the VAS, WOMAC, and Lequesne indices and resulted in displacement of the whole distribution toward smaller values, with decreased median values (P50%, Table 4). In the cell-treated group, 77% of the patients were satisfied or very satisfied with the treatment, whereas in the control group, this percentage fell to 38%.

Figure S2A (**SDC,** http://links.lww.com/TP/B133) shows knee pain relief at the 1-year follow-up, assessed by WOMAC (pain subscale), as a function of the initial pain score.[24] Treatment efficacy is equal to the slope of the line, with a slope of 1 indicating the "perfect treatment." Even though some scatter existed, a positive correlation was observed among values obtained in the MSC-treated group ($r = 0.74$; $P < 0.02$) around a line with a slope of 0.42 ± 0.10 (mean ± SE). In the controls treated with hyaluronic acid, the slope of the best-fit line was smaller, 0.10 ± 0.08. The difference between both slopes was statistically significant ($P < 0.02$), indicating that the treatment with cells was successful. The other indices reflected similar results, although the differences between the control and experimental groups were not always statistically significant, likely because of the dispersion of individual data. In Figure S2B (**SDC,** http://links.lww.com/TP/B133), we plotted the 3 main algofunctional indices: VAS, WOMAC (general), and Lequesne. We fitted a straight line to all control values on 1 side and to all experimental values on the other. This provided 2 lines with slopes of (mean ± SE) 0.14 ± 0.05 and 0.38 ± 0.06, respectively. The difference was highly significant ($P < 0.005$), confirming that the cell treatment relieved pain and disability.

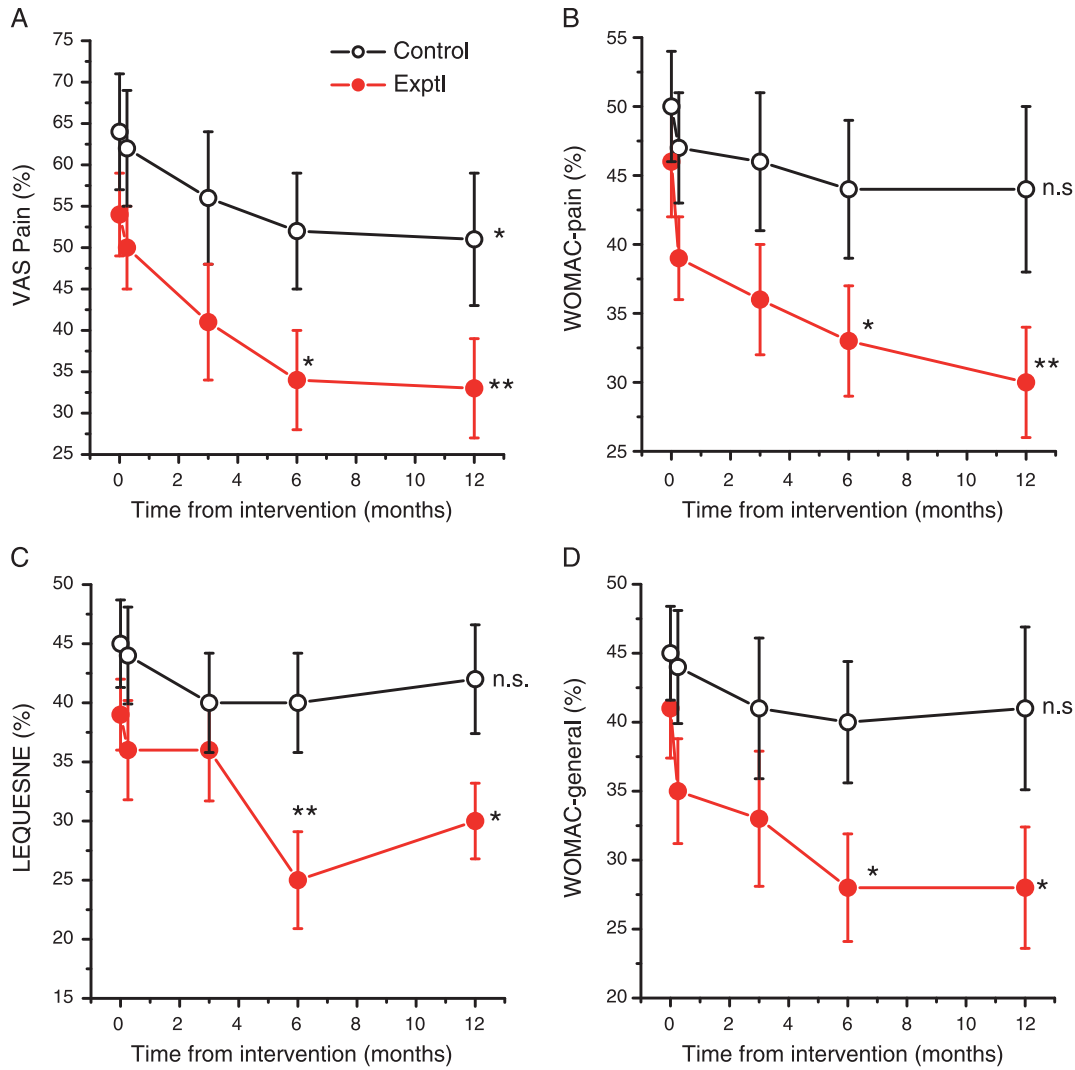

FIGURE 1. MSC-treatment resulted in improvements in pain and disability. A, The graph shows the evolution of knee pain over time, as measured by the VAS. Mean ± SE values are provided for the 15 patients treated with MSCs (closed symbols) and for the other 15 treated with the active control (hyaluronic acid; open symbols), $^{**}P < 0.01$, $^{***}P < 0.001$ (repeated-measures ANOVA with a Dunnet multiple test compared to the baseline, n.s. = non-significant ($P > 0.05$). B–D, The evolution of other algofunctional indices is shown. WOMAC-gen, WOMAC general scale; ANOVA, analyses of variance.

In our previous study with autologous cells, we did not find an effect on the life quality, as measured by the SF-36 questionnaire.[4] Consistent with these results, in the present study, we did not find significant differences between the control and the cell-treated patients in either the physical or mental component scores of the SF-12 questionnaire (**Table S1, SDC,** http://links.lww.com/TP/B133). However, the SF-36 questionnaire has been reported to be less sensitive for assessing knee arthritis than WOMAC, which was developed specifically for patients with lower extremity arthritis.[35] In fact, in several previous studies, the SF-36 scores were barely modified in either control or treated osteoarthritis patients.[36,37] Thus, we place more value on the WOMAC scoring system.

Imaging

The MRI quantitative T2 mapping has been used previously to evaluate articular cartilage quality. T2 relaxation time is sensitive to both changes in cartilage hydration and collagen fibril orientation.[32,38] T2 relaxation time is longer in remodeling inflammatory tissue versus hyaline cartilage[32,33] and increases in osteoarthritis.[38-40] Healing could then be followed by the decrease of the T2 relaxation time in affected areas. Figure 2A illustrates the decrease of T2 values in several areas of the articular cartilage after treatment with MSC in one of the patients. Note improvement after treatment in the areas indicated by white arrows. In order to perform a quantitative analysis, the articular cartilages were segmented in 88 ROIs (see details in Methods) and the mean T2 relaxations were computed. Figure 2B shows the transversal profile of an anteroposterior section and illustrates partial healing by treatment by MSC, with decrease of the mean T2 relaxation times in several ROIs.

In healthy knees, the mean T2 relaxation value of the 88 ROIs was (±SD) 37.0 ± 6.8 ms. Because 95% of values should be smaller than mean + 2 × SD, 50 (=37 + 2 × 6.8) was chosen as the threshold above which T2 values were considered inordinately high.[4] For this reason, the PCI was computed as the percentage of T2 values greater than 50 ms. A PCI of 100 is the worst possible value and a value near

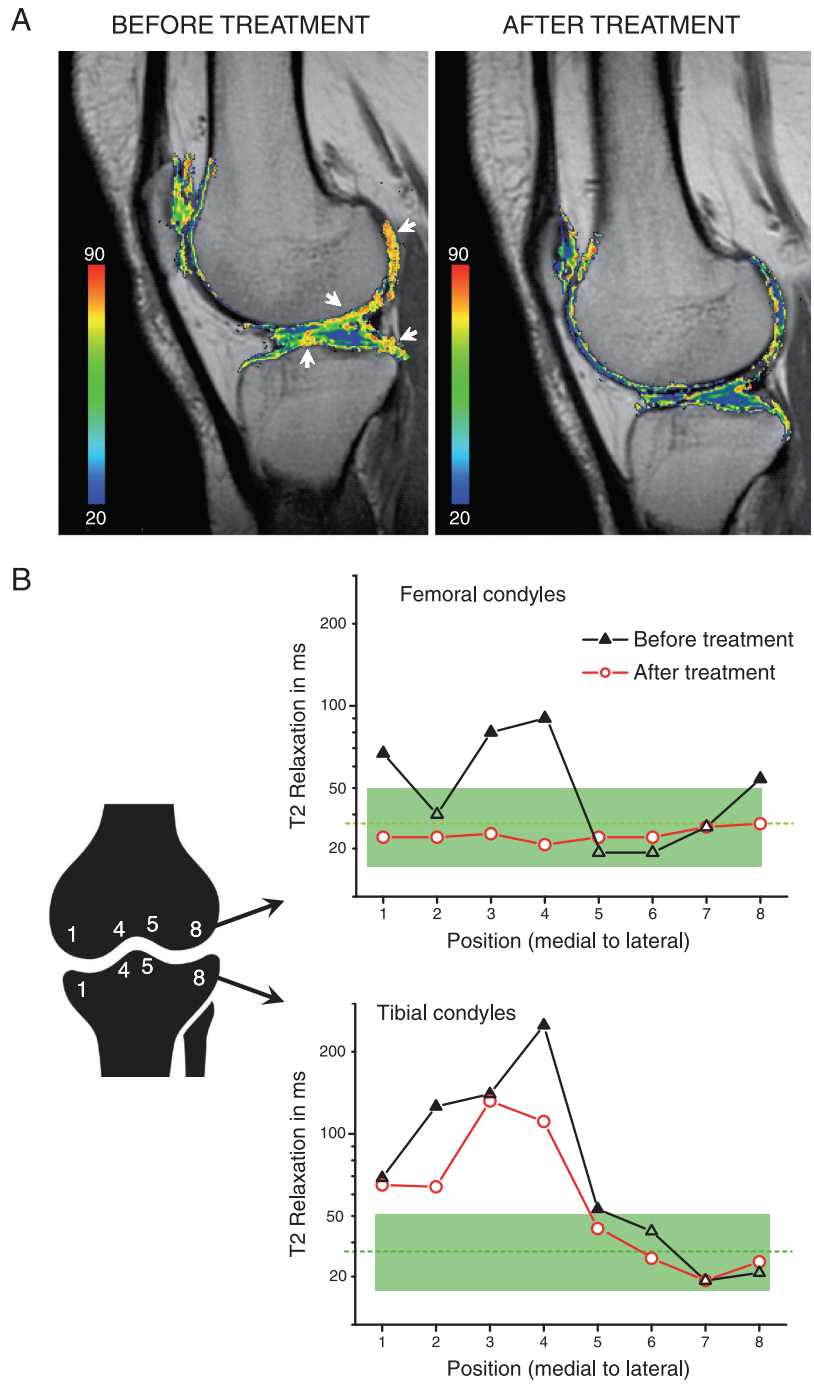

FIGURE 2. Monitorization of articular cartilage quality by T2 mapping. A, Sagittal view of external condyles (plane 7 of 22). T2 image is shown in grey. T2 relaxation measurements are superimposed in pseudocolor. Calibration scale at left (20 to 90 ms). Poor quality cartilage shows longer relaxation values, coded in red and yellow. Note improvement after treatment in the indicated areas (white arrows). B, Reconstruction of a transverse profile (at anteroposterior plane 2 of 4) of T2 relaxation times. Data were averaged in 8 manually segmented ROIs from medial to lateral condyles, as indicated. The values before and after the treatment are compared. Note logarithmic scale. The normal mean value is 37 ms and values over 50 ms are considered inordinately high. A green rectangle delimiting the normal values has been drawn in the figure. Black triangles correspond to baseline measurements before treatment whereas red circles represent measurements taken at the end of the assay. Note that 8 high values decreased, 4 of them to normal limits. In the total counting (88 ROIs) 11 of 21 were switched from greater than 50 to less than 50 ms by the treatment in this patient.

5 is considered healthy.[4] Figure 3A compares the evolution of the PCI in cell-treated patients versus the active controls treated with hyaluronic acid. The PCI decreased in both groups, but the decrease was not statistically significant in the control, whereas it reached significance ($P < 0.05$) in the experimental group at the 12-month follow up. In Figure 3B, PCI improvement was plotted against the baseline PCI score. In the treated patients, the slope of the line (efficiency of treatment) was 0.69, whereas in the control series, the slope was only 0.28. However, the differences were not significant as dispersion of the data was rather high (DS = 0.49 and 0.38, respectively). Both lines intercepted the abscissa axis near

TABLE 5.

Comparison of results of clinical trials with different cellular treatments of knee osteoarthritis

Clinical trial	Intervention[a]	Duration[b]	n	Basal[c]	+Treatment[c]	Pain relief[d]	Efficacy[e]	Effect size[f]
Orozco, 2013	Auto_BM_MSC	1 y	12	24 ± 14	6 ± 6	18 ± 13	0.75	1.29
Jo, 2014	Auto_AT_MSC	6 mo	12	56 ± 19	34 ± 23	22	0.39	0.96
This Study	Allo_BM_MSC	1 y	15	46 ± 15	30 ± 16	16 ± 23	0.36	1.07
This Study	Control (HA)	1 y	15	50 ± 14	44 ± 23	6 ± 16	0.12	0.43

[a] Auto_BM_MSC, Autologous Bone Marrow-derived Mesenchymal Stromal Cells. Auto_AT_MSC, Autologous Adipose Tissue-derived MSC. Allogeneic Bone Marrow-derived MSC. Control (HA), active control (hyaluronic acid).
[b] Duration in years or months.
[c] The WOMAC index (pain subscale) has been used; scale 0-100.
[d] Pain relief is computed as the difference between "basal" (initial pain score) and "treatment" values.
[e] Efficacy is computed as the quotient Pain Relief/Basal.
[f] Effect size is computed as Pain Relief/Basal SD.

the 5% value, which is the randomly expected value for non-affected individuals (Figure 3B).

DISCUSSION

Previous studies have demonstrated that autologous MSCs provide excellent therapeutic alternative for treating osteoarthritis[4-6]; however, allogeneic cells, which have been extensively tested for safety (see Introduction) would be logistically more convenient. This trial was, to the best of our knowledge, the first to use allogeneic MSC to test therapeutic efficacy in knee osteoarthritis. Our results show that allogeneic MSC transplantation is safe and feasible and results in no major adverse outcomes. Postimplantation pain was observed in 53% to 60% of patients (Table 3) and showed similar frequencies in the experimental and control groups. This pain responded well to ibuprofen, vanishing within 1 to 6 days. This minor adverse outcome was also reported at a similar frequency (50%) in a trial that tested autologous MSCs.[4]

Improvement of cartilage quality can be followed by MRI through T2 mapping, which focuses on the evolution of poor cartilage areas and filters out most of the spurious variation, thus enhancing the sensitivity of the procedure.[4] Cartilage healing can be conveniently followed by the decrease of the poor-quality cartilage areas, quantified by PCI. In our previous study, we showed that PCI improved significantly after treatment with autologous MSC and continued to improve through the second year follow-up.[5] We find here that allogeneic MSC treatment also significantly improves PCI (Figure 3), suggesting that these MSC aid in cartilage repair or regeneration. Adipose tissue–derived MSCs have also been reported to aid in cartilage repair, as evidenced by MRI and histological indications of hyaline cartilage regeneration.[6] Further investigation of the cartilage healing progression over a longer time period and investigation into the effect of repeated MSC application is warranted for future studies.

The analgesic effect of allogeneic MSC treatment is remarkable, resulting in 38% to 42% improvement in pain compared to 10% to 14% in active controls with hyaluronic acid (Figure 1A and B; **Figure S2A, SDC,** http://links.lww.com/TP/B133). Improvements in the other algofunctional indices were similar (Figure 1C and D; **Figure S2B, SDC,** http://links.lww.com/TP/B133).

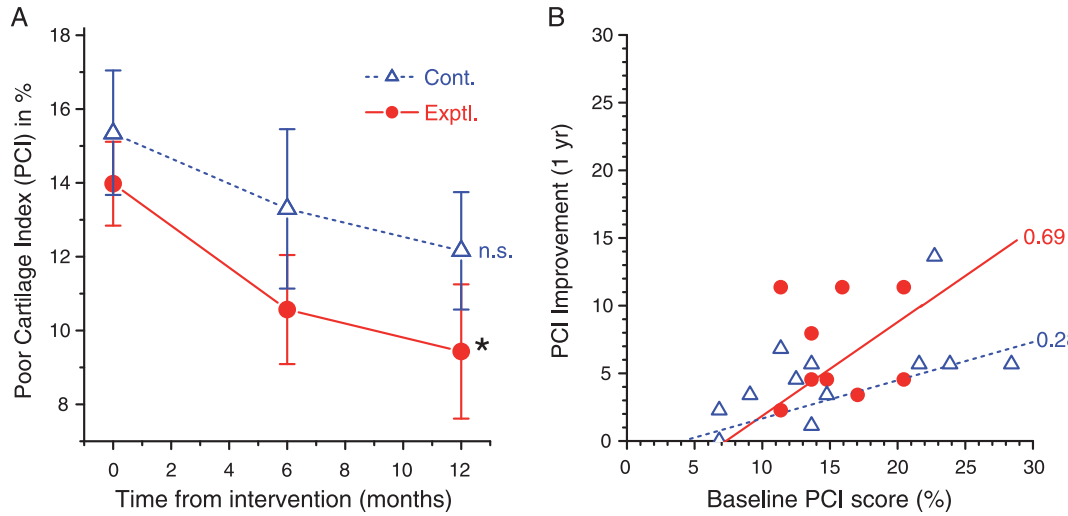

FIGURE 3. Cartilage quality improved as a result of MSC-treatment. Cartilage quality was assessed by MRI T2 mapping and was quantified using the PCI (computed as the percentage of sample points with a T2 relaxation value > 50 ms). The worst possible value for PCI is 100, whereas healthy cartilage will approach 5.[4] A, The graph shows the temporal evolution of PCI, mean ± SE values of 12 patients treated with MSCs (filled circles; continuous line) and 15 active controls treated with hyaluronic acid (open triangles; dotted line), *$P < 0.05$ (repeated measures ANOVA with a Dunnet multiple test compared to the baseline), n.s. = nonsignificant. B, The correlation between PCI improvement and the initial PCI score is shown for all the patients included in this study. Best-fit lines (not forced through the origin) are shown. The values of the slopes are shown on the right.

The effects were significant at 6 and 12 months for MSC-treated patients, whereas smaller improvements in the active control group were, in general, not statistically significant. The efficacy of the allogeneic MSC treatment for pain relief compares favorably to the reported for conventional treatments. For example, in a recent meta-analysis of 4 prestigious clinical trials, we find an efficacy range of 4% to 36 % for 8 different treatments (see Figure 3 in Orozco et al[4]). Table 5 compares the results obtained here with allogeneic cells to those of previous trials using autologous MSC. Efficacy was quantified for comparisons of different trials by dividing pain relief by the initial pain score.[24] This gives values between 0 (no effect) and 1 (perfect treatment). Values of *effect size*, a primary measurement of significance (see Table 4 legend), are also shown. The efficacy of allogeneic treatment found in this trial was smaller than that reported for autologous BM -derived cells[4] and similar to that reported with autologous adipose tissue-derived cells[6] (Table 5); however, direct comparisons are difficult because the previous studies were uncontrolled. Apart from differences in the cell type, the fact that the present study was a multicenter endeavor that included two different surgical teams may have contributed to differences by increasing variability. In fact, it would be interesting to directly compare autologous with allogeneic cells in different arms of the same trial, and we are moving in this direction for future studies.

The results published here are the first to demonstrate feasibility and safety of allogeneic MSCs while providing strong indications for their efficacy in treating osteoarthritis. These results may contribute to more widespread use of MSC treatments because allogeneic cells are cheaper and more logistically convenient than autologous cells. Additionally, studies are increasingly demonstrating the biosafety of allogeneic MSCs (see Introduction), which also encourages clinical use. However, the transition from autologous to allogeneic MSCs should be made with extreme caution to ensure safety. Allogeneic MSC treatments will benefit from further research addressing how MSCs relieve pain, promote regeneration, and become immune evasive. In addition, protecting MSCs from immune detection and prolonging their persistence in vivo may improve clinical outcomes and prevent sensitization.[8] Research that has been performed suggests that, due to the fast acting nature of MSC treatments, many of the early beneficial effects may be trophic by promoting immunomodulatory[7,41] and/or anti-inflammatory effects.[42] Importantly, MSCs have also been shown to stimulate cocultured cells to proliferate and synthesize extracellular matrix.[43-45] In fact, transplanted MSCs engrafted 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.[46] Because of these *hit and run* effects, tracing MSC action may be elusive.

In summary, we propose that cell therapy with expanded allogeneic BM-MSC be considered as a treatment for chronic osteoarthritis. Cell handling and expansion of these cells is reproducible, and quality control tests are satisfactory. The clinical procedure is feasible and safe, and requires minimally invasive intervention without surgery or hospitalization. The procedure results in significant relief of pain and disability, and quantitative MRI evidence indicates partial articular cartilage healing. Advantages of allogeneic over autologous treatments include lower cost, higher homogeneity, and the possibility of using them in seropositive patients. The healing effects appear to be somewhat smaller than those reported for treatment with autologous MSC, but this should be confirmed in future studies designed to directly compare both cell types within the same trial.

ACKNOWLEDGMENTS

The authors thank Mr. Jesús Fernández, Ms. Sandra Güemes, and Ms. Virginia Gordillo from IBGM, and Ms. Carmen Barbero from ITRT for technical support. Durolane (Bioventus; 60 mg of hyaluronic acid in a 3 mL) was generously provided by Dr. A. Esteras, Director of Medical Marketing, Zambon S. A.U., Spain.

REFERENCES

1. Arden N, Nevitt MC. Osteoarthritis: epidemiology. *Best Pract Res Clin Rheumatol*. 2006;20:3–25.
2. Hawker GA, Mian S, Bednis K, Stanaitis I. Osteoarthritis year 2010 in review: non-pharmacologic therapy. *Osteoarthritis Cartilage*. 2011;19:366–374.
3. McAlindon TE, Bannuru RR, Sullivan MC, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthritis Cartilage*. 2014;22:363.
4. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. *Transplantation*. 2013;95:1535–1541.
5. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: two-year follow-up results. *Transplantation*. 2014;97:e66–e68.
6. Jo CH, Lee YG, Shin WH, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells*. 2014;32:1254–1266.
7. Le Blanc K, Ringden O. Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med*. 2007;262:509–525.
8. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol*. 2014;32:252.
9. Chang CH, Kuo TF, Lin FH, et al. Tissue engineering-based cartilage repair with mesenchymal stem cells in a porcine model. *J Orthop Res*. 2011;29:1874–1880.
10. Dashtdar H, Rothan HA, Tay T, et al. A preliminary study comparing the use of allogenic chondrogenic pre-differentiated and undifferentiated mesenchymal stem cells for the repair of full thickness articular cartilage defects in rabbits. *J Orthop Res*. 2011;29:1336–1342.
11. Shimomura K, Ando W, Tateishi K, et al. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. *Biomaterials*. 2010;31:8004–8011.
12. Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy*. 2007;23:178–187.
13. Muschler GF, Nakamoto C, Griffith LG. Engineering principles of clinical cell-based tissue engineering. *J Bone Joint Surg Am*. 2004;86-A:1541–1558.
14. Wang D, Zhang H, Liang J, et al. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. *Cell Transplant*. 2013;22:2267–2277.
15. Koc ON, Gerson SL, Cooper BW, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol*. 2000;18:307–316.
16. Hare JM, Fishman JE, Gerstenblith G, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA*. 2012;308:2369–2379.
17. Wang P, Li Y, Huang L, et al. Effects and safety of allogenic mesenchymal stem cells intravenous infusion in active ankylosing spondylitis patients who failed NSAIDs: a 20 week clinical trial. *Cell Transplant*. 2013;23:1293–1303.
18. Lazarus HM, Koc ON, Devine SM, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant*. 2005;11:389–398.
19. Bernardo ME, Pagliara D, Locatelli F. Mesenchymal stromal cell therapy: a revolution in Regenerative Medicine? *Bone Marrow Transplant*. 2012; 47:164–171.

20. Peeters CM, Leijs MJ, Reijman M, van Osch GJ, Bos PK. Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: a systematic literature review. *Osteoarthritis Cartilage*. 2013;21:1465–1473.
21. Orozco L, Soler R, Morera C, Alberca M, Sanchez A, Garcia-Sancho J. Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. *Transplantation*. 2011;92:822–828.
22. Bannuru RR, Natov NS, Dasi UR, Schmid CH, McAlindon TE. Therapeutic trajectory following intra-articular hyaluronic acid injection in knee osteoarthritis—meta-analysis. *Osteoarthritis Cartilage*. 2011;19:611–619.
23. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*. 1957;16:494–502.
24. Huskisson EC. Measurement of pain. *Lancet*. 1974;2:1127–1131.
25. Lopez Alonso SR, Martinez Sanchez CM, Romero Canadillas AB, Navarro Casado F, Gonzalez Rojo J. Metric properties of WOMAC questionnaires-original and reduced versions-to measure symptoms and physical functional disability. *Aten Primaria*. 2009;41:613–620.
26. Faucher M, Poiraudeau S, Lefevre-Colau MM, Rannou F, Fermanian J, Revel M. Assessment of the test-retest reliability and construct validity of a modified WOMAC index in knee osteoarthritis. *Joint Bone Spine*. 2004;71:121–127.
27. Gandek B, Ware JE, Aaronson NK, et al. Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: results from the IQOLA Project. International Quality of Life Assessment. *J Clin Epidemiol*. 1998;51:1171–1178.
28. Roth WK. Quarantine plasma: quo vadis? *Transfus Med Hemother*. 2010;37:118–122.
29. Blanco JF, Graciani IF, Sanchez-Guijo FM, et al. Isolation and characterization of mesenchymal stromal cells from human degenerated nucleus pulposus: comparison with bone marrow mesenchymal stromal cells from the same subjects. *Spine*. 2010;35:2259–2265.
30. 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.
31. Battaglia M, Vannini F, Buda R, et al. Arthroscopic autologous chondrocyte implantation in osteochondral lesions of the talus: mid-term T2-mapping MRI evaluation. *Knee Surg Sports Traumatol Arthrosc*. 2011;19:1376–1384.
32. Battaglia M, Rimondi E, Monti C, et al. Validity of T2 mapping in characterization of the regeneration tissue by bone marrow derived cell transplantation in osteochondral lesions of the ankle. *Eur J Radiol*. 2011;80:e132–e139.
33. Giannini S, Battaglia M, Buda R, Cavallo M, Ruffilli A, Vannini F. Surgical treatment of osteochondral lesions of the talus by open-field autologous chondrocyte implantation: a 10-year follow-up clinical and magnetic resonance imaging T2-mapping evaluation. *Am J Sports Med*. 2009;37(Suppl 1):112S–118S.
34. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. L. Erlbaum Associates: Hillsdale, NJ; 1988.
35. Hawker G, Melfi C, Paul J, Green R, Bombardier C. Comparison of a generic (SF-36) and a disease specific (WOMAC) (Western Ontario and McMaster Universities Osteoarthritis Index) instrument in the measurement of outcomes after knee replacement surgery. *J Rheumatol*. 1995;22:1193–1196.
36. Kirkley A, Birmingham TB, Litchfield RB, et al. A randomized trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med*. 2008;359:1097–1107.
37. Witt C, Brinkhaus B, Jena S, et al. Acupuncture in patients with osteoarthritis of the knee: a randomised trial. *Lancet*. 2005;366:136–143.
38. Crema MD, Roemer FW, Marra MD, et al. Articular cartilage in the knee: current MR imaging techniques and applications in clinical practice and research. *Radiographics*. 2011;31:37–61.
39. 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;232:592–598.
40. Kim HK, Laor T, Graham TB, et al. T2 relaxation time changes in distal femoral articular cartilage in children with juvenile idiopathic arthritis: a 3-year longitudinal study. *AJR Am J Roentgenol*. 2010;195:1021–1025.
41. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005;105:1815–1822.
42. Yang SH, Wu CC, Shih TT, Sun YH, Lin FH. In vitro study on interaction between human nucleus pulposus cells and mesenchymal stem cells through paracrine stimulation. *Spine*. 2008;33:1951–1957.
43. Acharya C, Adesida A, Zajac P, et al. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol*. 2012;227:88–97.
44. Qing C, Wei-ding C, Wei-min F. Co-culture of chondrocytes and bone marrow mesenchymal stem cells in vitro enhances the expression of cartilaginous extracellular matrix components. *Braz J Med Biol Res*. 2011;44:303–310.
45. Wu L, Prins HJ, Helder MN, van Blitterswijk CA, Karperien M. Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. *Tissue Eng Part A*. 2012;18:1542–1551.
46. Horie M, Choi H, Lee RH, et al. Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. *Osteoarthritis Cartilage*. 2012;20:1197–1207.