SUPPLEMENTAL DIGITAL CONTENT (SDC)

Contents:

Table S1. Antecedent history of the patients included in this trial.

Pat. Num.	Sex	Age	Side	OA Grade	Previous Surgery	RHB	NSAID	Corti-coids	Hyaluronic Acid	PRP Num. (Date)	Date MSV
1	F	66	L	IV		Yes	Yes			3 (2007) 3 (2008)	2010
2	M	41	R	III	ACL+MM (1991) MCL (2004)	Yes	Yes				2010
3	M	44	R	II	MM (2009) ACL (2009)	Yes	Yes				2010
4	F	41	L	III	LM (2001)	Yes	Yes	3 (2002) 3 (2003)			2010
5	F	35	R	II	ACL (1991) MM (2001) LM(2010)	Yes	Yes				2010
6	M	33	L	II	ACL (1998) MM (2007) MM(2009)	Yes	Yes			4 (2010)	2010

7	M	29	R	II	ACL (2001) MM + LM (2007)	Yes	Yes		3 (2009)		2010
8	M	43	L	IV	LM (2001)	Yes	Yes			3 (2003) 3 (2006) 3(2007)	2010
9	F	39	R	III	ACL+MM (1991)	Yes	Yes	2 (2010)			2010
10	M	75	R	IV		Yes	Yes	3 (2005) 3 (2006)			2011
11	M	71	L	IV		Yes	Yes	3 (2007)		3 (2010)	2011
12	F	72	L	IV	OSTEOT. (2000)	Yes	Yes		2 (2007)	4 (2009) 4 (2010)	2011

OA, Osteoarthritis; RHB, Rehabilitation; NSAID, non-steroidal antiinflammatory drug; Cortic., Infiltration with corticosteroids; PRP, Platelet-rich plasma; MSV, Mesenchymal Stem Cells; ACL, Anterior cruciate ligament; MM, Medial meniscus; LM, Lateral meniscus; MCL, medial collateral ligament; OSTEOT., Osteotomy (tibial).

Table S2. Minor adverse events

Minor adverse event (Comments)	***Participants affected (%)***
Post-implantation pain at days 1-6 (E, SR)	6/12 (50%)
Articular inflammation attributable to knee overloading (E, PSR)	3/12 (25%)
Unexpected knee inflammation with synovial fluid effusion and articular swelling (UE, PSR)	3/12 (25%)
Low back pain (UE, PSR)	3/12 (25%)
Pain in the contralateral knee (UE, PSR)	1/12 (8%)
Ischiotibial tendonitis (UE, PSR)	1/12 (8%)
Arthroscopic surgery in the contralateral knee (UE, NSR)	1/12 (8%)
Dental implant (UE, NSR)	1/12 (8%)
Influenza (UE, NSR)	1/12 (8%)
Intolerance to gluten and and to lactose (UE, NSR)	1/12 (8%)

Comments: (E) Expected; (UE) Unexpected; (SR) Study-Related; (NSR) Not Study-Related; (PSR) Possibly Study-Related.

In all cases, the adverse events responded to medical/physical therapy.

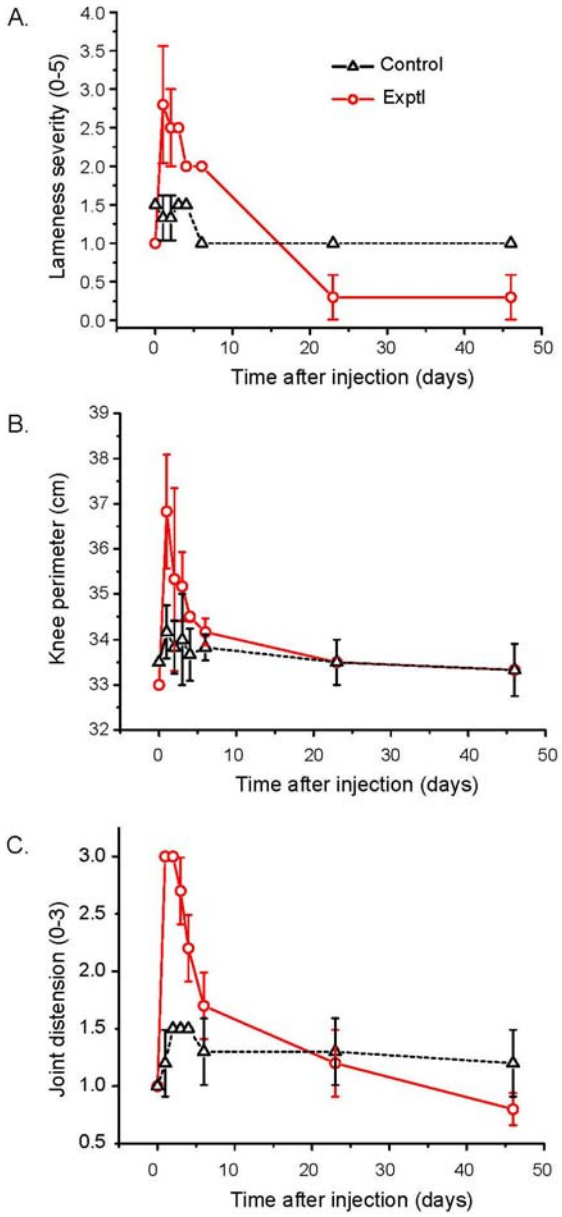
Table S3. Meta-analysis of clinical trials with different osteoarthritis treatments and comparison of their efficacies.

Clinical Trial	Intervention[a]	Duration[b]	n	Basal[c]	+Treatment[c]	Improvement[d]	Impr./Basal slope[e]	Code[f]
Pisters et al., 2010 (15)	BGA	1 yr	55-75	43±14	31	13	0.29	1
	UC	1 yr	51-70	43±13	29	13	0.31	2
	BGA	5 yr	55-75	43±14	31	12	0.28	-
	UC	5 yr	51-70	43±13	34	8	0.19	-
Kirkley *et al.*, 2008 (13)	CONT	3 m	86	43±23	34±22	9	0.20	-
	SURG	3 m	92	49±20	31±20	19	0.37	-
	CONT	2 yr	86	43±23	37±24	6	0.13	-
	SURG	2 yr	92	49±20	36±26	13	0.26	-
	CONT	1 yr	86	43±23	31±21	12	0.28	3
	SURG	1 yr	92	49±20	34±24	16	0.32	4
Witt *et al.*, 2005 (14)	ACCUP	2 m	149	51±19	27±1	24	0.47	-
	SHAM	2 m	75	53±19	36±2	17	0.32	-
	ACCUP	1 yr	149	51±19	33±22	18	0.36	5

	SHAM	1 yr	75	53±19	38±23	14	0.27	6
Moseley *et al.,* 2002 (10)	PCB	0.5 m	59	60±19	48±24	12	0.19	-
	LAV	0.5 m	61	59±17	52±20	7	0.12	-
	DEBR	0.5 m	58	59±22	53±22	6	0.10	-
	PCB	2 yr	59	60±19	53±25	7	0.10	7
	LAV	2 yr	61	59±17	57±24	3	0.04	8
	DEBR	2 yr	58	59±22	54±23	5	0.09	9
This Study, 2012	MSCs	1 yr	12	24±14	6±6	18	0·78	•

[a]BGA, Behavior-Graded Activity (no drugs); UC, Usual Care (only physical; no drugs); ACCUP, acupuncture (non-steroidal anti-inflammatories as needed; compares with sham-treated); PCB, Placebo; LAV, Lavage (medical treatment as needed); DEBR, Debridement (medical treatment as needed); CONT, control; SURG, surgery (lavage plus debridement; physical and medical therapy as required); MSCs, Mesenchymal stem cells expanded from bone marrow samples in the current study shown here. The Western Ontario and McMaster Universities Arthritis (WOMAC) index (pain component) has been used; scale 0-100. [b]Duration in years (yr) or months (m); [c]Where appropriate, mean±SD is given; [d]Difference between “basal” and “treatment” values. [e]Impr./Basal slope, ratio improvement/basal = slope in Figure 3; [f]Code in Figure 3.

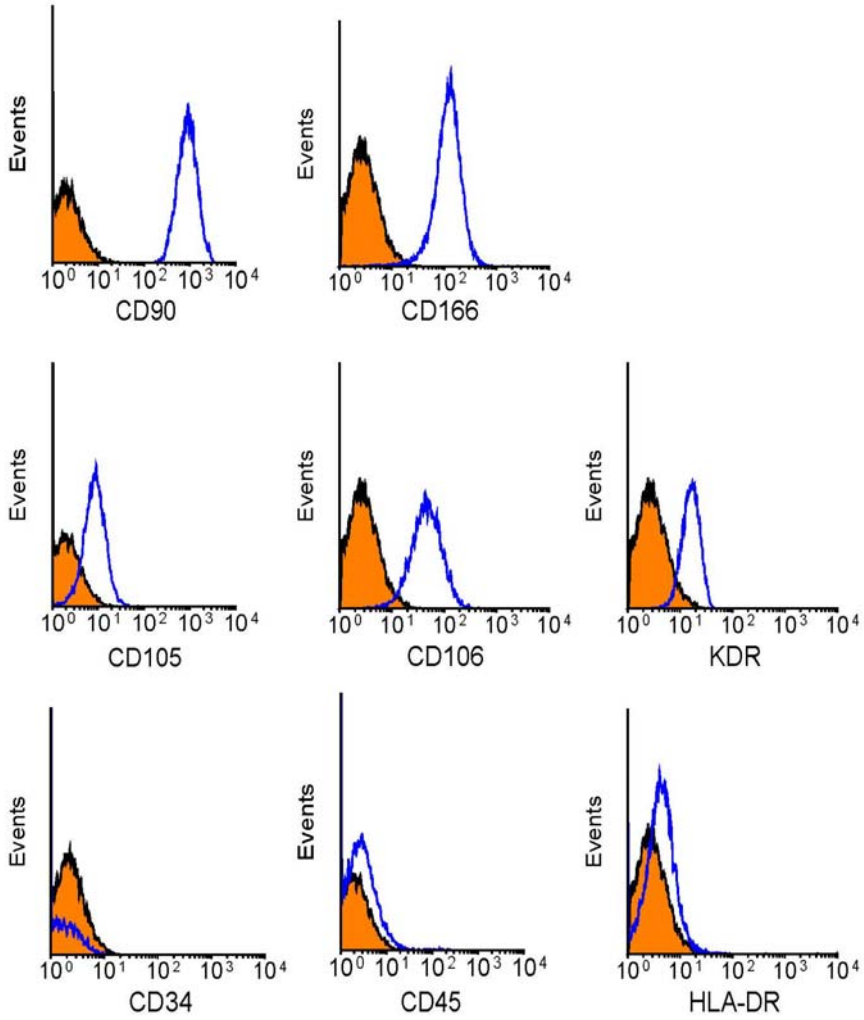
Figure S1. Effects of MSCs in horses.



The intended clinical protocol was first tested in three horses to assess feasibility and safety. Experiments in horses were approved by the Autonomous University of Barcelona's Animal Care and Use Committee. The tibiotarsal joint was chosen for the experiments. Limited lesions (1.5×1.5 cm laterally, and 4 mm deep, avoiding the subchondral plate) were produced in trochlea tali of both posterior legs. Autologous MSCs were prepared from

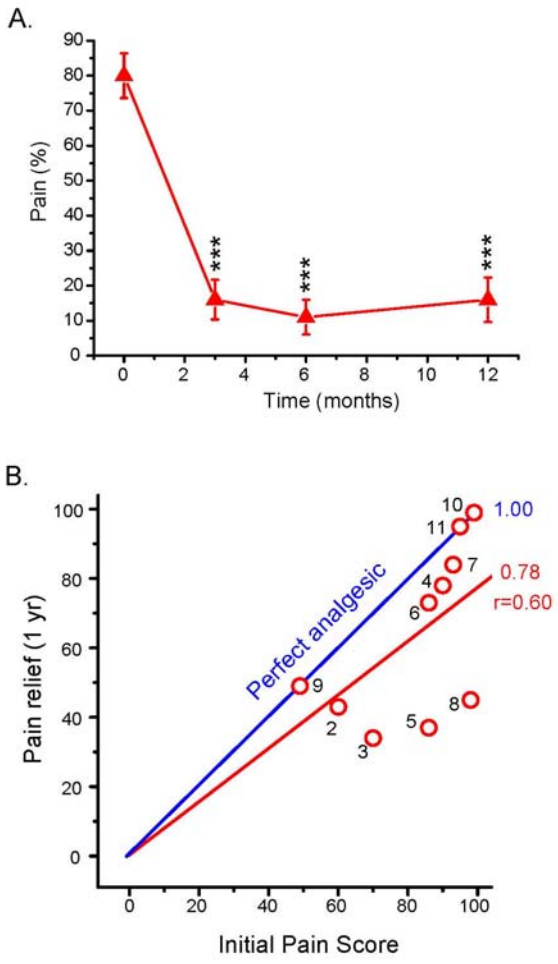
approximately 80-ml sternum bone marrow samples, which were expanded for 21 days to obtain 50×10^6 MSCs, following the same protocol used in humans (see *Methods*). Cells were suspended in 10 ml of autologous plasma and injected intra-articularly 2 weeks after the lesion was created. A volume of 10 ml of MSC suspension was injected into one joint and 10 ml of vehicle (phosphate-buffered saline) into the contralateral joint. The follow-up period was 46 days. The horses were maintained in stalls during the first 3 days and then in a 10×10 m fenced space for 43 additional days. Clinical tests were performed at the times shown and included quantification of **(A)** lameness severity (0-5 scale), **(B)** knee diameter, and **(C)** joint distension (0-3 scale, appreciated by palpation). The values shown are the mean ± SE of three independent experiments. The lesion produced lameness and inflammation, estimated from knee diameter and joint distension. Injection of MSCs produced considerable additional inflammation and worsened lameness during the first 1-2 days. Then symptoms declined slowly during the whole observation period with a half-time period of 6-12 days. In controls injected with saline the inflammatory peak was much smaller. By the end of the observation period, inflammation was less in the joints injected with MSCs. The necropsy, performed at the end of the 6 months period, did not reveal local nor general alterations. Overall, results supported feasibility and safety of the procedure. Our team also performed a preliminary study in 10 sheep, in which a limited lesion was generated in the femoral condyles and the internal meniscus. Five sheep were injected with 8 ml of saline as controls and the other five animals received the same solution containing 50×106 autologous bone marrow MSCs. We observed clear regeneration of cartilage and the meniscus in the MSC-treated sheep at 12 months post-treatment compared to no improvement in control animals. Necropsy did not reveal other local or general alterations (unpublished results by R. Soler and L. Orozco).

Figure S2. Immunophenotypic characterization of MSCs.



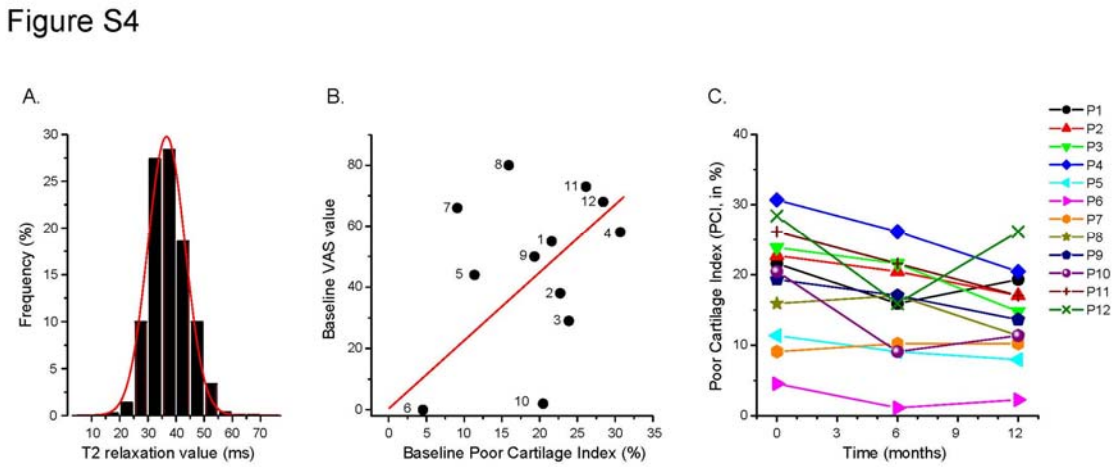
Flow cytometric analysis of MSCs (blue) compared with isotype controls (orange). MSCs were strongly positive for CD90 and CD166; moderately positive for CD105, CD106, and KDR; and negative for CD34, CD45, and HLA-DR. Representative results are shown.

Figure S3. **Effects of MSC on sports activity-associated pain.**



A. Graph showing evolution of knee pain associated to sports activity, as measured by VAS (VAS-SA), over time. Mean ± standard error (SE) values of 8 patients treated with MSC. Data from 4 patients were not included because these series were not complete. ***p<0.001 (ANOVA; Bonferroni test for paired values). **B.** Correlation between improvement of knee pain 1 year after treatment with MSCs and initial pain score, as measured with VAS-SA. The "perfect" treatment (dotted line with slope of 1) is shown for comparison. The best-fitting line is shown with values for the slope and linear regression coefficient (r) at the right. The figures besides data points are the patient codes. Patients 4 and 8 were not included because data were incomplete.

Figure S4. T2 mapping results.



A. Distribution of the T2 relaxation values (ms) obtained in nine measurements in healthy individuals; 88 areas were analyzed in each knee articulation: 24 in the patella, 32 in the femoral condyles, and 32 in the tibial condyles. Mean±SD = 39.0±6.8 (n=792). Percentile 95=50; Gaussian fitting is also shown (r=0.984). **B.** Correlation between baseline values of Poor Cartilage Index (PCI) and VAS. PCI was computed as the percentage of T2 relaxation readings >50 ms. Numbers beside data points correspond to patient codes. The best-fitting line is also shown. Linear regression analysis: r=0.34; p<0.001. **C.** Temporal evolution of PCI in each individual patient. Codes as in Panel B.