Intervertebral Disc Repair by Allogeneic Mesenchymal Bone Marrow Cells: A Randomized Controlled Trial

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Background. Degenerative disc disease often causes severe low-back pain, a public health problem with huge economic and life quality impact. Chronic cases often require surgery, which may lead to biomechanical problems and accelerated degeneration of the adjacent segments. Autologous mesenchymal stromal cells (MSC) treatments have shown feasibility, safety and strong indications of clinical efficacy. We present here a randomized, controlled trial using allogeneic MSC, which are logistically more convenient than autologous cells. **Methods.** We randomized 24 patients with chronic back pain diagnosed with lumbar disk degeneration and unresponsive to conservative treatments into 2 groups. The test group received allogeneic bone marrow MSCs by intradiscal injection of 25×10^6 cells per segment under local anesthesia. The control group received a sham infiltration of paravertebral musculature with the anesthetic. Clinical outcomes were followed up for 1 year and included evaluation of pain, disability, and quality of life. Disc quality was followed up by magnetic resonance imaging. **Results.** Feasibility and safety were confirmed and indications of clinical efficacy were identified. MSC-treated patients displayed a quick and significant improvement in algofunctional indices versus the controls. This improvement seemed restricted to a group of responders that included 40% of the cohort. Degeneration, quantified by Pfirrmann grading, improved in the MSC-treated patients and worsened in the controls. **Conclusions.** Allogeneic MSC therapy may be a valid alternative for the treatment of degenerative disc disease that is more logistically convenient than the autologous MSC treatment. The intervention is simple, does not require surgery, provides pain relief, and significantly improves disc quality.

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Intervertebral disc degeneration is a very common disease that can lead to axial skeletal pain, radiculopathy, and myelopathy. Combined physical and medical therapies are successful in relieving pain in approximately 90% of the cases. However, the remaining 10% become chronic and generate a serious public health problem, as chronic low-back pain ruins both the life quality and the labor capacity of the patient, and increases use of health services.[1,2]

The efficacy of the existing treatments is limited (see our recent meta-analysis).[3] Surgery is the recommended criterion standard when the back pain chronifies and its analgesic value is beyond question,[1,4] but it has several drawbacks.[5,6] Cell therapy has produced exciting results both in vitro and in vivo,[7] and animal studies with mesenchymal stem cells (MSC) have been particularly promising.[8] On the other hand, in a recent pilot trial in humans, treatment with

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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. D.C.N., A.S., and J.G.S. participated in the conception and design of the article. D.C.N., F.A., R.H.R., and M.A.M.F. were primarily responsible for the clinical work, and A.S. and M.A. and V.G. for the cell production. I.S.L. and B.T. were responsible for M.R.I., J.M.M. provided advice. All authors participated in analysis, discussion and interpretation of data, revision of the article, and gave final approval of the version to be published. J.G.S. put together all data, did meta-analysis and wrote the final form of the article.

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autologous MSC produced strong indications of clinical efficacy, with pain improvement approaching 71% of optimal during the first year. In addition, although disc height was not recovered, water content was significantly elevated.[3] This outcome compares favorably with the results of other interventions such as spinal fusion or total disc replacement, suggesting that MSC treatment could be a valid alternative treatment for chronic back pain caused by degenerative disc disease (DDD).

Even though autologous MSCs are an excellent therapeutic option, the need for cell expansion makes the procedure slow and expensive. Allogeneic cells would be logistically, much more convenient, but they may have important drawbacks, the most obvious the possibility of host immune rejection of the transplanted cells. MSC, however, are *immune privileged*[9] or *immune evasive*[10] and inhibit immune responses in a manner not restricted by the HLA system. As a result, nonmatched MSC are much better tolerated than other cell types. In fact, there are no reports of rejection in animal experiments[8,11-14] and studies of transplanted MSC persistence in the host organism show the same values for autologous and allogeneic cells.[10,15] 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.[16] Similarly, no transplantation-related adverse events occurred in MSC-treated patients with breast cancer,[17] left ventricular dysfunction,[18-20] ankylosing spondylitis,[21] graft versus host disease,[22] and other autoimmune diseases.[23] We have demonstrated recently the safety of autologous MSC for treatment of knee osteoarthritis,[24] an application very similar to the one proposed in the present paper.

Here, we present a randomized controlled study to assess the feasibility and safety of using good manufacturing practice (GMP)-compliant bone marrow MSCs.[3,25] Additionally, we present evidence to suggest that intradiscal injection of allogeneic cells has a therapeutic value. The intervention proposed does not require surgery, does not produce anatomical modifications, and does not hinder further interventions, should they be required.

MATERIALS AND METHODS

Patients and Procedures

This phase I-II trial was approved by Ethics Committee at Valladolid University Hospital and by the Spanish Agency of Medicines (EudraCT 2012-004444-30). The study was also registered at ClinicalTrials.gov (NCT01860417). The design of the trial was based on our previous one, performed with autologous MSC.[3] We recruited 24 patients (17 men and 7 women; mean age ± SE = 38±2 years) with Pfirrmann grade II-IV DDD [26], that had been unresponsive to conventional treatments (physical and medical) for at least 6 months before recruitment. Recruitment was performed between July 2013 and March 2014. Detailed inclusion and exclusion criteria are reported in Table 1. 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. Clinical, analytical, and imaging evaluations were performed to ensure compliance with the inclusion criteria.

TABLE 1.

Inclusion and exclusion criteria

Inclusion criteria
1. Degenerative disease of 1 or 2 lumbar discs with predominant back pain after conservative treatment (physical and medical) for over 6 months
2. Fibrous ring capable of holding the cell implantation, demonstrated by MRI (stages 2, 3, and 4 of Pfirrmann).
3. Decrease of disc height of more than 20 % (radiographic measurement in side image).
4. Absence of spinal infection.
5. Haematological and biochemical analysis with no significant alterations that contraindicate intervention.
6. The patient is able to understand the nature of the study.
7. Informed written consent of the patient.
8. In fertile women, negative pregnancy test result and acceptance of adequate contraceptive methods
Exclusion criteria
1. Age, older than 75 yr or younger than 18 yr or legally dependent
2. Allergy to gentamicin, or to bovine, cattle or horse serum.
3. Congenital or acquired diseases leading to spine deformations that may upset cell application.
4. Spinal segmental instability, spinal canal stenosis, isthmus pathology and other conditions that may compromise the study
5. Modic III changes on MRI.[27]
6. Overweight with body mass index (mass in kg/size in m^2) greater than 35 (obesity grade II)
7. Pregnancy or breastfeeding
8. Neoplasia
9. Immunosuppression
10. Hypersensitivity to amide-type local anaesthetics or other known contraindications or interactions of mepivacaine
11. Participation in another clinical trial or treatment with another investigational product within 30 days before inclusion in the study.
12. Other conditions that may, according to medical criteria, discourage participation in the study.

Patients were informed of the protocol design before providing written informed consent.

Follow-Up Controls

The protocol included 6 visits (V0-V5). The V0 visit involved a final compliance check using the inclusion criteria, performance of the complementary evaluations and tests needed, and scheduling of dates for the next visit. At V1, treatments were administered, either MSCs (25×10^6 MSC in 2 mL of saline per disc) under local anesthesia or sham infiltration of paravertebral musculature close to the affected disc(s) with 2 mL of 1% mepivacaine. The V2-V5 visits (8 days, and 3, 6, and 12 months after implantation) included clinical evaluation and routine analyses, pain evaluation using visual analogue scale (VAS),[28] Oswestry Disability Index (ODI),[29] and short form-12 (SF-12) life quality questionnaire.[30] Outcomes were expressed using a 0% to 100% scale in all cases. Quantitative magnetic resonance imaging (MRI) exploration was performed at V0, V4, and V5. The patients, radiologists, care providers, and persons assessing the outcomes of the assay were blinded after assignment.

Cell Isolation and Expansion

Bone marrow was obtained from 5 healthy donors and processed using GMP conditions in the IBGM Cell Production

Unit as described previously.[3,25] Isolations were carried out with the following parameters (mean ± SD; n = 5, 4 men and 1 woman): bone marrow volume = 105 ± 5 mL, average number of mononuclear cells obtained = $1.23 \pm 0.25 \times 10^9$, expansion time = 27 ± 2 days, number of MSC injected into each disc = 25×10^6, suspended in Ringer-lactate at 12.5×10^6 cells/mL, and viability greater than 98% ± 1%. A serum sample from each donor was obtained to screen for human immunodeficiency, hepatitis B, and hepatitis C virus by nucleic acid amplification technology.[31] The cells obtained from each donor were used for 1 to 3 recipients. Immune matching was not attempted.

Statistics

Data are reported as mean ± SD or SE, as indicated in each case. Significant differences were assessed by either Student *t* tests, by 2-way repeated-measures analyses of variance, or with appropriate corresponding nonparametric tests. We used GraphPad Instat3 package software version 3.06 (GraphPad Software, La Jolla, CA) for all calculations.

RESULTS

Patients

This pilot study included 24 patients (see details in Materials and methods) diagnosed of DDD with preserved external annulus fibrous and persistent low-back pain. Additionally, all 24 patients did not respond to conservative treatment (physical and medical) lasting at least 6 months. The patients had 1 or 2 affected discs, with the lesion located at L1-L2 (n = 1), L2-L3 (1), L3-L4 (3), L4-L5 (18), or L5-S1 (15). They were informed of the protocol design before providing written informed consent. The protocol included 6 visits (V0-V5), as described in Materials and Methods. All patients were treated at the Valladolid University Hospital. No major adverse events occurred. Eleven patients (8 controls and 3 cell-treated) required brief treatments with nonsteroidal anti-inflammatory drugs-type analgesics for minor pains and 2 (1 control and 1 cell-treated) required opioids (morphine sulphate tablets or tramadol).

Evolution of Pain, Disability, and Quality of Life

Table 2 summarizes the distribution of pain and disability indexes throughout the observation period. The baseline values of pain and disability were quite homogeneous in the cohort. On average patients felt intense lumbar pain (65 ± 5 in the VAS scale) and had moderate disability (ODI of 29 ± 4) (mean ± SE; n = 24). Both lumbar pain and disability were significantly reduced at 3 months after MSC transplantation, and the improvement was maintained at 6 and 12 months (Figures 1A and C). Compared with the basal level of pain and disability, improvement was statistically significant at all time points except at 8 days (see details in Figure 1, legend). The pattern of improvement between VAS and ODI was parallel and resulted in global displacement of the whole distribution towards smaller values, with a strong decrease of the

TABLE 2.

Total sum score of VAS measurements for lumbar pain and ODI

VAS_CONT	C or E	N	MEAN	DS	SE	SMD	MIN	P25%	P50%	P75%	MAX	Conf 95	Conf 99
V0	C	12	62	23	7	N.A.	20	56	71	77	82	13	17
V2 (8 d)	C	12	45	25	7	0.75	9	22	49	61	81	14	19
V3 (3 mo)	C	12	46	27	8	0.71	0	24	50	62	85	15	20
V4 (6 mo)	C	12	51	29	8	0.49	6	26	52	79	84	17	22
V (12 mo)	C	12	47	28	8	0.67	2	24	54	68	87	16	21
VAS_EXPTL	C or E	N	MEAN	DS	SE	SMD	MIN	P25%	P50%	P75%	MAX	Conf 95	Conf 99
V0	E	12	67	26	7	N.A.	30	50	70	90	100	15	19
V2 (8 d)	E	12	63	26	7	0.17	16	51	67	82	99	14	19
V3 (3 mo)	E	12	43	30	9	0.94	7	16	40	63	98	17	23
V4 (6 mo)	E	12	40	29	8	1.07	3	12	47	60	93	17	22
V (12 mo)	E	12	47	36	10	0.80	3	14	47	78	95	21	27
ODI_CONT	C or E	N	MEAN	DS	SE	SMD	MIN	P25%	P50%	P75%	MAX	Conf 95	Conf 99
V0	C	12	24	14	4	N.A.	4	15	22	30	46	8	10
V2 (8 d)	C	12	20	16	5	0.30	0	10	16	31	50	9	12
V3 (3 mo)	C	12	25	15	4	–0.10	2	16	24	31	52	8	11
V4(6 mo)	C	12	30	20	6	–0.43	2	14	28	45	60	11	15
V(12 mo)	C	12	34	25	7	–0.76	2	20	29	51	94	14	19
ODI_EXPTL	C or E	N	MEAN	DS	SE	SMD	MIN	P25%	P50%	P75%	MAX	Conf 95	Conf 99
V0	E	12	34	23	7	N.A.	2	22	26	47	78	13	17
V2 (8 d)	E	12	27	17	5	0.31	2	18	21	29	60	10	13
V3 (3 mo)	E	12	16	20	6	0.76	2	6	9	16	72	11	15
V4(6 mo)	E	12	20	24	7	0.62	0	7	12	19	88	13	18
V(12 M)	E	12	22	24	7	0.53	0	8	10	24	72	14	18

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

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 Cohen.[32]

In all cases, the scale was from 0% to 100%. Measurements performed before cell transplantation (baseline), 8 days, 3, 6 and 12 months afterward (12 months) are shown.

Conf 95 and Conf 99, confidence interval at 95% and 99% significance; min, minimum value; max, maximum value; SMD, standardized mean difference, computed as improvement (baseline value minus value at the end of treatment) divided by the SD of the baseline value; C, control patients (mepivacaine); E, experimental cell-treated patients; N.A., does not apply.

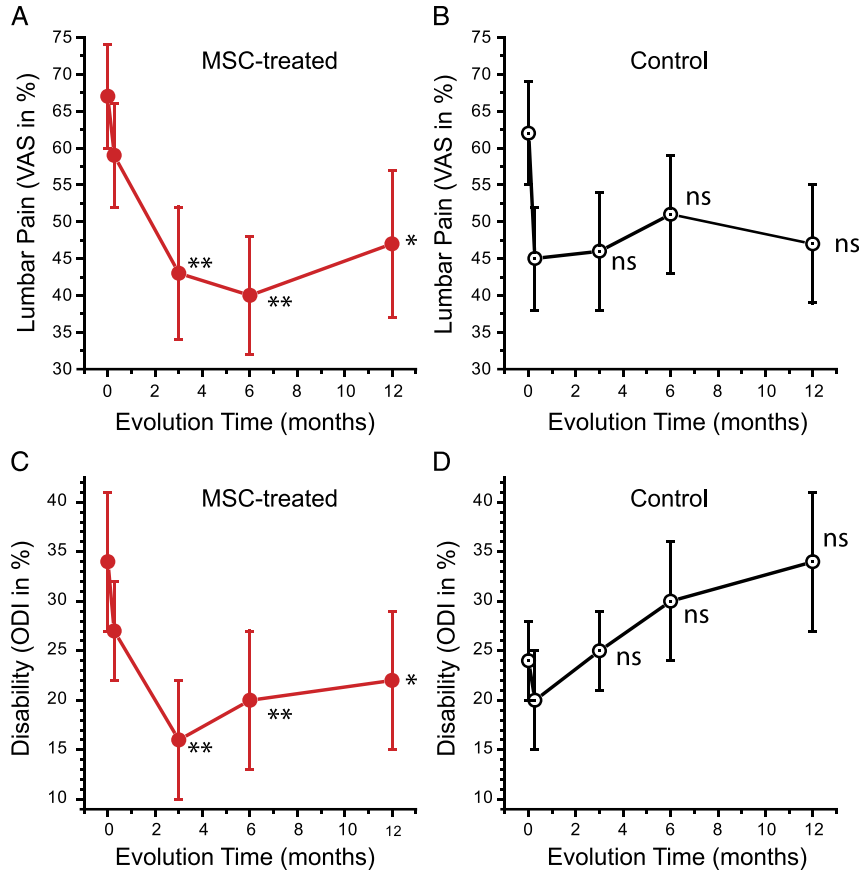

FIGURE 1. Temporal evolution of pain and disability over time after MSC treatment. A, B, Graph showing changes of lumbar pain over time for MSC-treated patients (A) and control patients (B). VAS, Visual Analog Scale. C, D, Graph showing changes of disability over time as measured by ODI for MSC-treated (C) and control patients (D). Represented values are mean ± SE. Statistical significance assessed by ANOVA for paired populations, Bonferroni test. Comparisons to $t = 0$; ns, nonsignificant, $^{*}P < 0.05$; $^{**}P < 0.01$. ANOVA, analysis of variance.

medians (P50% in Table 2; see also **Figure S1, SDC**, http://links.lww.com/TP/B347). Note that the effect was virtually complete at the third month. In the sham-treated controls the effects at 3, 6, and 12 months were not statistically significant. A fast decrease of pain was detected at the eighth day in the control group, but there was no tendency for further improvement thereafter (**Figures 1B and D, and Figure S1B, SDC**, http://links.lww.com/TP/B347). The ODI values tended to increase at 3, 6, and 12 moths in the control group (Figure 1D).

In Figure 2, we have plotted lumbar pain relief at the end of the treatment, assessed by VAS, as a function of the initial pain score[28] (Figure 2, circles). As shown before,[3] the improvement of the disability index as a function of the baseline ODI value exhibited the same relationship and can be included in the same plot (Figure 2, triangles). The efficacy of the treatment is equal to the slope of the dotted lines, fitted either for the control patients (open symbols, in black) or for the cell-treated patients (filled symbols, in red). The "perfect treatment" with a slope of 1 is also shown (continuous line, in blue). The controls fitted to a line with slope of (mean ± SEM) 0.15 ± 0.10, not significantly different from 0 (p = 0.13), whereas the cell-treated patients fitted to a line with slope 0.28 ± 0.07, which is significantly different from 0 ($P < 0.001$). There was considerable scatter not only in the control group but also in the experimental group. In the last case, however the cohort seemed to divide into 2 groups, 1 group of 5 patients close to the blue line, with a high relief index (responders) and the remaining patients, which show little improvement (nonresponders).

In our previous study with autologous MSCs, we found a slight but significant improvement of the physical component score in the SF-36 quality of life questionnaire.[3] Although this tendency was also observed in the present work, the SF-12 life quality questionnaire did not reveal significant improvements of either the physical or the mental component scores (**Table S1; SDC**, http://links.lww.com/TP/B347). This is not surprising, because these indices are less sensitive than the pain tests in the inflammatory diseases.[25,33,34]

Imaging

MRI was used to assess disc height and water content of the discs. The height of the affected discs decreased by (in mm; mean ± SE) 0.38 ± 0.19 mm the controls (n = 16 discs) and by only 0.04 ± 0.19 mm in the cell-treated patients (n = 17 discs), but the difference was not significant. In neighbor healthy discs (n = 24), the heights decreased by 0.07 ± 0.10 mm (n = 24 discs). These values were not significantly different. Water content of the discs, determined from T2-weighted sagittal images, was measured in the affected disc segment and in the contiguous 3 to 5 segments (see *Methods* and Orozco et al[3]). In some patients the water content of the affected discs improved after treatment with the MSC with little changes of the normal discs (**Figure S2,**

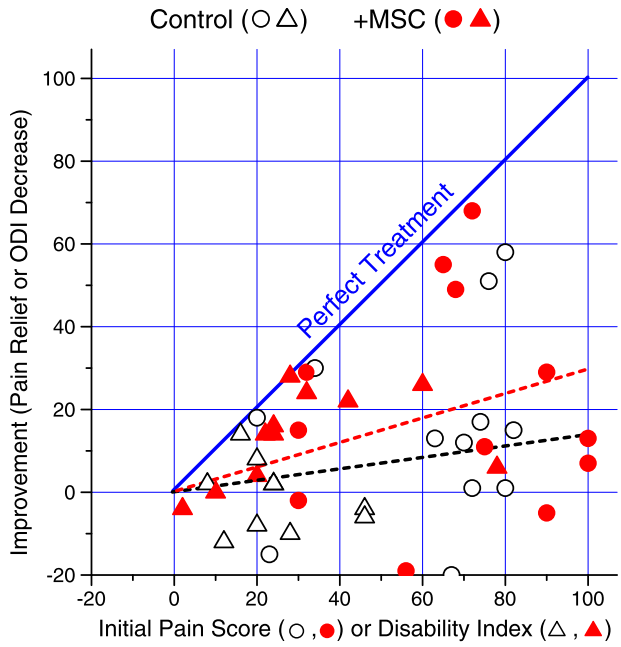

FIGURE 2. Pain and disability improvement as a result of MSC treatment. Improvement 1 year after intervention is plotted as a function of the initial pain score or disability index.[28] Results for the relief of lumbar pain (circles) and Oswestry disability index (triangles) are all included for both, control (open symbols) and cell-treated patients (filled symbols) The continuous blue line with a slope of 1 represents the perfect treatment, in which complete pain or disability relief was achieved. The dotted lines correspond to the linear fit of controls (black) and treated data (red). The values of the slopes obtained from the best fit of the data (n = 24; linear regression forced through the origin) were (mean ± SE) were: control, 015 ± 0.10 (not significantly different from 0; $P = 0.13$), and MSC-treated 0.28 ± 0.07 (significantly different from 0; $P < 0.001$).

SDC, http://links.lww.com/TP/B347). To homogenize the results of different patients, the water content values of the affected discs were normalized to the values obtained from the healthy discs in the same individual; for these purposes, the density of the affected segments was divided by the average value of the healthy discs. Finally, the value after the treatment was divided by the baseline value. In the cell-treated discs the image density increased by (mean ± SE) 22 ± 11 % at 12 months, compared to only 6% ± 8 % in controls (**Figure S3; Table S2, SDC**, http://links.lww.com/TP/B347). The differences, however, were not statistically significant ($P < 0.07$; 1-sample paired *t* test, 2-tailed value). In our previous trial with autologous MSC, the difference was very similar, 18% increase in water content, but it was statistically significant because the dispersion was smaller.[3] Evolution of Pfirrmann staging, which takes into account several MRI disc parameters,[26] was clearly different in the control and in the experimental groups. Results are shown in Figures 3A and B. In controls (B), there was a deterioration from (mean ± SEM; n = 20) Pfirrmann stage 3.15 ± 0.15 to stage 3.78 ± 0.16 ($P < 0.001$; Wilcoxon matched-pairs signed-ranks test), whereas in the cell-treated patients (A), there was an improvement from stage 3.68 ± 0.13 to 3.18 ± 0.17 ($P < 0.01$).

DISCUSSION

Previous studies have demonstrated that autologous MSCs provide an excellent therapeutic alternative for treating DDD [3]; however, allogeneic cells, which have been extensive and successfully tested for safety (see Introduction), would be logistically much more convenient than autologous for these treatments. This trial is, to the best of our knowledge, the first to test therapeutic efficacy of allogeneic MSC in DDD. Our results show that allogeneic MSC transplantation is feasible and safe, and does not originate any major adverse outcome. Occasional mild pain reactions were, if anything, more frequent in the control than in the cell-treated patients and responded well to the usual analgesic treatments.

The parallel improvement of pain and disability effected by the MSC treatment was quick but not immediate; it reached about 30% of the maximum at 8 days after the intervention, was nearly complete at 3 months and was maintained at 6 and 12 months (Figure 1). In the controls not treated with MSC the time course was very different. There was a sudden decrease of VAS during the first 8 days, but no further improvement along the following year was observed. As a matter of fact, the disability index worsened (although not statistically significantly) along the first year after the intervention. We do not know the reason for this early improvement of pain in the controls; it could be due to a placebo effect or be the result from the anesthetic infiltration, but in any of these cases, it should also happen in the cell-treated group, and there is not indication of this extra fast early improvement in the group of cell-treated patients.

Efficacy of the treatments was quantified by the slope of the pain relief-initial pain score relationship.[28] This gives values between 0 (no effect) and 1 (perfect treatment). Values of *effect size*, a primary measurement of significance (see Table 2 legend), were also computed. There was a clear analgesic effect of the allogeneic MSC, resulting in an average 28% improvement in pain and disability 1 year after the intervention. This compares with only 15% recovery in the sham-treated controls (Figure 2). The improvement was

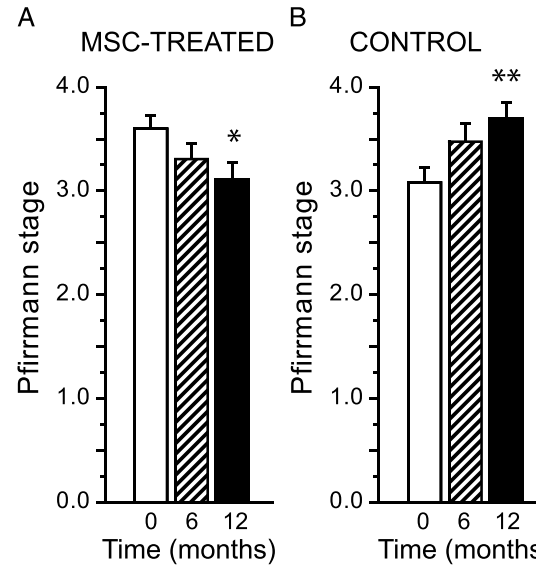

FIGURE 3. Assessment of nucleus pulposus evolution by Pfirrmann grading. Pfirrmann grading (1 to 5) takes into account the structure of the disc, the distinction of nucleus pulposus and annulus fibrosus, the signal intensity and the height of the disc. The values (mean ± SE) at baseline (open) or 6 months (crosshatched) and 12 months after treatment (filled) are shown for control (B) and MSC-treated patients (A). Comparisons to baseline were performed by repeated measures ANOVA, Bonferroni multiple comparisons; *$P < 0.01$; **$P < 0.001$.

statistically significant in the cell-treated group but not in the control group.

Quantification of the slope of the pain relief/baseline pain score relationship permits comparison of efficacy among different trials.[28] The efficacy of allogeneic treatment found in the present trial, 0.28, was smaller than the reported for autologous cells, 0.71[3]; yet, direct comparisons are difficult because the previous study was uncontrolled. In fact, it would be most 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.

Close inspection of Figure 2 is suggestive of bimodal distribution of the cell-treated patients in the Huskisson plot; a responders subgroup of 5 patients is close to the blue line that represents perfect treatment, whereas the other 7 (nonresponders) distribute very similarly to the control patients, with no indication of effectiveness. If we were able to understand the reasons for this different behavior, that might help to understand the action mechanism of the healing effect and, eventually, to improve efficacy by choosing the most adequate cells among different available donors.

In any case, the results published here are the first to demonstrate the feasibility and safety of the allogeneic MSCs treatment while providing also indications for their efficacy for relief of chronic low back pain. As allogeneic cells are more logistically convenient than autologous, the results presented here may contribute to more widespread use of MSC treatments. In addition all the studies coincide on the safety 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, not only clinical, but also basic, addressing how MSCs relieve pain, promote regeneration, and become immune evasive.[10] In recent times the importance of HLA matching to determine kidney allograft survival has been questioned.[35-37] However, a recently published exhaustive investigation (189 141 cohort) concludes that every mismatch between donor and recipient increases risk by 11% (hazard ratio from 1.13 for 1 mismatch to 1.64 for 6 mismatches).[38] The present case is not comparable because the accessibility inside the disc is severely limited and, as pointed out above, MSC are not only immune evasive but do also depress immune reactions (see Introduction). In any case, it would be very interesting to investigate the relationship between HLA mismatch and efficiency in the patients treated with allogeneic MSC in a further study.

We can only speculate regarding the mechanism by which the beneficial effect of the treatment occurs. Animal studies have shown that MSC injected in the NP area are able to survive and proliferate[39,40] and to induce beneficial effects in DDD.[41,42] Nucleus pulposus cells induce differentiation of cocultured MSC into nucleus pulposus-like cells with a chondrocyte phenotype.[43-45] Even more importantly, MSC stimulated nucleus pulposus cells to proliferate and to synthesise extracellular matrix.[46,47] This action may be important in vivo as very few MSC are required to trigger this effect.[47] In addition, MSC display a well-known immunomodulatory effect and express Fas-ligand when implanted in the spinal discs of dogs.[41] These data indicate that MSC may help analgesia by reducing inflammation. Additionally, MSC can induce the production of anti-inflammatory cytokines.[47,48] Importantly, MSCs have also been shown to stimulate cocultured cells to proliferate and synthesize extracellular matrix.[49-51] In fact, transplanted MSCs engrafted into the joint in mouse 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.[52] Because of these *hit and run* effects, tracing MSC action may be elusive.

In summary, we propose that cell therapy with expanded allogeneic bone marrow-derived MSC should be considered as a putative treatment for chronic DDD. GMP 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 suggests partial disc 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 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. These studies will track the long-term evolution as well as investigate the anatomical and functional changes that occur in the intervertebral spaces and will increase the number of patients, which is an important limitation of the present study.

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REFERENCES

1. Errico TJ, Gatchel RJ, Schofferman J, et al. A fair and balanced view of spine fusion surgery. *Spine J*. 2004;4(5 Suppl):S129–S138.
2. Balagué F, Mannion AF, Pellisé F, et al. Clinical update: low back pain. *Lancet*. 2007;369:726–728.
3. Orozco L, Soler R, Morera C, et al. Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. *Transplantation*. 2011;92:822–828.
4. Kwon B, Katz JN, Kim DH, et al. A review of the 2001 Volvo Award winner in clinical studies: lumbar fusion versus nonsurgical treatment for chronic low back pain: a multicenter randomized controlled trial from the Swedish lumbar spine study group. *Spine (Phila Pa 1976)*. 2006;31:245–249.
5. Harrop JS, Youssef JA, Maltenfort M, et al. Lumbar adjacent segment degeneration and disease after arthrodesis and total disc arthroplasty. *Spine (Phila Pa 1976)*. 2008;33:1701–1707.
6. Rihn JA, Lawrence J, Gates C, et al. Adjacent segment disease after cervical spine fusion. *Instr Course Lect*. 2009;58:747–756.
7. Fassett DR, Kurd MF, Vaccaro AR. Biologic solutions for degenerative disk disease. *J Spinal DisordTech*. 2009;22:297–308.
8. Yim RL, Lee JT, Bow CH, et al. A systematic review of the safety and efficacy of mesenchymal stem cells for disc degeneration: insights and future directions for regenerative therapeutics. *Stem Cells Dev*. 2014;23:2553–2567.
9. Le Blanc K, Ringdén O. Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med*. 2007;262:509–525.
10. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol*. 2014;32:252–260.
11. 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;29:1874–80.
12. 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.

13. 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.
14. Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy*. 2007;23:178–187.
15. Muschler GF, Nakamoto C, Griffith LG. Engineering principles of clinical cell-based tissue engineering. *J Bone Joint Surg Am*. 2004;86-A:1541–1558.
16. 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.
17. Koç 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.
18. 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.
19. Perin EC, Borow KM, Silva GV, et al. A Phase II dose-escalation study of allogeneic mesenchymal precursor cells in patients with ischemic or nonischemic heart failure. *Circ Res*. 2015;117:576–584.
20. Karantalis V, Hare JM. Use of mesenchymal stem cells for therapy of cardiac disease. *Circ Res*. 2015;116:1413–1430.
21. 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;23:1293–303.
22. 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.
23. Bernardo ME, Pagliara D, Locatelli F. Mesenchymal stromal cell therapy: a revolution in Regenerative Medicine? *Bone Marrow Transplant*. 2012;47: 164–171.
24. Vega A, Martin-Ferrero MA, Del Canto F, et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial. *Transplantation*. 2015;99:1681–1690.
25. 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.
26. Niu G, Yang J, Wang R, et al. MR imaging assessment of lumbar intervertebral disk degeneration and age-related changes: apparent diffusion coefficient versus T2 quantitation. *AJNR Am J Neuroradiol*. 2011;32: 1617–1623.
27. Modic MT, Ross JS. Lumbar degenerative disk disease. *Radiology*. 2007; 245:43–61.
28. Huskisson EC. Measurement of pain. *Lancet*. 1974;2:1127–1131.
29. Davidson M, Keating JL. A comparison of five low back disability questionnaires: reliability and responsiveness. *PhysTher*. 2002;82:8–24.
30. 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.
31. Roth WK. Quarantine plasma: quo vadis? *Transfus Med Hemother*. 2010; 37:118–122.
32. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale, NJ: L Erlbaum Associates; 1988.
33. Witt C, Brinkhaus B, Jena S, et al. Acupuncture in patients with osteoarthritis of the knee: a randomised trial. *Lancet*. 2005;366:136–143.
34. 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.
35. Ashby VB, Port FK, Wolfe RA, et al. Transplanting kidneys without points for HLA-B matching: consequences of the policy change. *Am J Transplant*. 2011;11:1712–1718.
36. Morales JM, Marcén R, Andrés A, et al. Renal transplantation in the modern immunosuppressive era in Spain: four-year results from a multicenter database focus on post-transplant cardiovascular disease. *Kidney Int Suppl*. 2008:S94–99.
37. Su X, Zenios SA, Chakkera H, et al. Diminishing significance of HLA matching in kidney transplantation. *Am J Transplant*. 2004;4:1501–1508.
38. Williams RC, Opelz G, McGarvey CJ, et al. The risk of transplant failure with HLA mismatch in first adult kidney allografts from deceased donors. *Transplantation*. 2016;100:1094–1102.
39. Henriksson HB, Svanvik T, Jonsson M, et al. Transplantation of human mesenchymal stems cells into intervertebral discs in a xenogeneic porcine model. *Spine (Phila Pa 1976)*. 2009;34:141–148.
40. Sakai D, Mochida J, Iwashina T, et al. Differentiation of mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. *Spine*. 2005;30: 2379–2387.
41. Hiyama A, Mochida J, Iwashina T, et al. Transplantation of mesenchymal stem cells in a canine disc degeneration model. *J OrthopRes*. 2008;26: 589–600.
42. Sakai D, Mochida J, Iwashina T, et al. Regenerative effects of transplanting mesenchymal stem cells embedded in atelocollagen to the degenerated intervertebral disc. *Biomaterials*. 2006;27:335–345.
43. Risbud MV, Albert TJ, Guttapalli A, et al. Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype in vitro: implications for cell-based transplantation therapy. *Spine (Phila Pa 1976)*. 2004; 29:2627–2632.
44. Vadala G, Studer RK, Sowa G, et al. Coculture of bone marrow mesenchymal stem cells and nucleus pulposus cells modulate gene expression profile without cell fusion. *Spine (Phila Pa 1976)*. 2008;33:870–876.
45. Le Maitre CL, Baird P, Freemont AJ, et al. An in vitro study investigating the survival and phenotype of mesenchymal stem cells following injection into nucleus pulposus tissue. *Arthritis Res Ther*. 2009;11:R20.
46. Watanabe T, Sakai D, Yamamoto Y, et al. Human nucleus pulposus cells significantly enhanced biological properties in a coculture system with direct cell-to-cell contact with autologous mesenchymal stem cells. *J OrthopRes*. 2009;28:623–630.
47. Yang SH, Wu CC, Shih TT, et al. In vitro study on interaction between human nucleus pulposus cells and mesenchymal stem cells through paracrine stimulation. *Spine (Phila Pa 1976)*. 2008;33:1951–1957.
48. Krock E, Rosenzweig DH, Haglund L. The inflammatory milieu of the degenerate disc: is mesenchymal stem cell-based therapy for intervertebral disc repair a feasible approach? *Curr Stem Cell Res Ther*. 2015;10:317–328.
49. 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.
50. 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.
51. Wu L, Prins HJ, Helder MN, et al. 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.
52. 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.